# IMPACT OF GROWING LOCATION AND HARVEST TIME ON HEALTH PROMOTING COMPOUNDS FROM DANDELION LEAFY GREEN

A Thesis

by

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# ABSTRACT

Dandelion is considered a weed and undervalued by most people. Dandelion's bitter sensory appeal and lack of knowledge for its health benefits offsets its potential to be consumed as a leafy green. The focus of this study investigated the impact of growing location, harvest time and determined which leafy green part acquired high levels of health promoting compounds from various dandelion varieties.

Dandelion var. Catalonga leaf blade had higher amounts of vitamin C, carotenoids, chlorophyll A and chlorophyll B, antioxidant activity and dietary fiber versus the whole leaf and petiole during the late harvest. Catalonga from New Jersey had highest amounts of vitamin C, Catalonga from Texas and New Jersey contained violaxanthin, lutein, β-carotene, chlorophyll A and B. DPPH scavenging activity and total phenolic content was highest in the leaf blade of Catalonga Texas. Catalonga Texas had a higher percentage of bound bile acid salts, CDCA (sodium chenodeoxycholate) and DCA (sodium deoxycholate), and total dietary fiber.

In continuation, various dandelion varieties were treated with different thermal processing techniques to acquire hot aqueous extracts and tested for antioxidant potential. Dandelion var. Garnet Stem from Texas, resulted in high DPPH scavenging activities when boiled for 15 min and microwaved for 4 min. Both boiling and microwaving contained highest antioxidant activity versus hot sonication. Lastly, successive extraction of phenolics decreased drastically as the time of boiling increased

but dandelion varieties were able to recover almost at the same level of phenolics when boiled for 15 min.

Results from thermal extraction presented significant amounts of antioxidant activity from Garnet Stem and was furthered investigated. Garnet Stem's red midvein/petiole tissue was used in an optimized extraction protocol and four anthocyanins were quantified for the first time, cyanidin-3-(6-malonyl)-glucoside (A-2) with the greatest amount present. Vitamin C and β-carotene were highest in the leaf blade of Garnet Stem from New Jersey. Lutein, violaxanthin, chlorophyll a, and chlorophyll b was the highest in leaf blade of Garnet Stem from Texas. Highest bound bile acid salt was CDCA and Garnet Stem from New Jersey contained the highest amount of total dietary fiber (40.5 %).

Both Catalonga and Garnet Stem varieties have different amounts of phytochemicals dependent on location and time of harvest in the leafy blade, but the presence of such health benefitting compounds proposes its use as another leafy green vegetable in the market. Dandelion leafy greens can be eaten fresh, raw or in an herbal tea form.

# DEDICATION

This thesis is dedicated to my family and to Anthony John Gigliotti for supporting my decision to attend Texas A&M University, for all of the encouragement they have given me while pursuing my master's degree and for all the strength they have bestowed within myself to never give up.

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# CONTRIBUTORS AND FUNDING SOURCES

## Contributors

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All work for the thesis was completed by the student, in collaboration Dr. Jashbir Singh of the Department of Horticultural Sciences. All other work conducted for the thesis was completed by the student independently.

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TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

# CHAPTER I

# INTRODUCTION AND LITERATURE REVIEW

## Introduction

Dandelion (*Taraxacaum officinale*) is a part of the *Asteraceae* family and has been known for its uses as an herbal medicine, depending on the locale, to improve human health.[1] The Native Americans boiled the dandelion plant in water and consumed the infused boiled dandelion water to relieve from "stomach upset, heart burn, skin problems, swelling and kidney disease."[2] The Chinese prepared the dandelion as decoctions to treat "stomach problems, appendicitis, and inflammation."[2] Based on location and culture, the dandelion was used differently for its curative abilities. Different parts of the plant provides multitude health benefits based on the phytochemicals present in dandelion.[1, 3, 4] Dandelion root suppresses the appetite and acts as a diuretic, the leaves are hepatoprotective due to the polyphenols and leaves help with indigestion, heart health and may reduce blood pressure.[3, 5-9]

Different varieties of dandelion have unique physical characteristics. Catalonga is a dark leafy green variety of dandelion grown in Mediterranean countries including Spain.[10] Garnet Stem variety of dandelion, a dark leafy green but contains a red stem and midrib, containing antioxidant anthocyanins.[11, 12] Previous studies have demonstrated certain health promoting properties due to dandelion's unique food composition and phytochemicals including: dietary fiber, vitamin C, β-carotene, lutein, chlorophyll and

nitrates, minerals (phosphorus, magnesium, calcium,), phenolics and flavonoids (cichoric acid, chlorogenic acid, caffeic acid, luteolin and quercetin).[13-16]

The dandelion plant can be used in different ways, as a food source, to obtain its health promoting compounds. The dandelion leaves can be eaten fresh in a salad or sautéed with butter, bacon or other types of fats.[5] The dandelion leaves and/or roots can be boiled to make tea and made into juices which may enhance bioavailability, depending on duration of boiling time or by type of processing during juicing.[17, 18] Dandelion, unfortunately, has limited availability in the food market but is widely abundant in the wild as a foraging plant.[1, 19]

## Literature Review

### *Dandelion background*

Dandelion (*Taraxacum officinale*) is a ubiquitous leafy green perennial plant and grows all over the United States in many warmer climate foraging areas, road sides, and residential areas.[1, 20, 21] Dandelion, a part of the *Asteraceae* family, has been used for many years in various locations as a medicinal plant to treat different health issues by Native Americans, Europeans in Mediterranean countries and Asian countries.[1, 3, 21] Dandelion is utilized by different cultures in various forms. Dandelion leaves are used by Spaniards and Italians to aid with stomach problems and dandelion roots are used by Asian countries to promote liver health.[1] This historical evidences suggest that phytochemicals in dandelion vary by growing locations.[1] Different sample parts of the dandelion plant (roots or leaves) may have distinctive amounts of specific phytochemicals and properties that may reduce the risk of certain diseases and illnesses, suggesting the health benefits of

dandelion may depend on the part consumed.[22] Roots and leaves have been used for diuretic aid, roots for anti-inflammatory and antimicrobial, and leaves for anti-hepatotoxicity, liver disorder, hypolipidemic, choleretic, cholesterol, heart disease and antioxidant activity.[4, 22-25]

The form of consumption of dandelion varies from culture to culture. A common form to consume dandelion is by boiling the leaves and/or roots due to sensory appeal. Dandelion is bitter in taste because of the presence of certain bioactive compounds: sequiterpene lactones, taraxinic acid β-D-glucopyranoside, 11, 13-dihydrotaraxinic-acid β-D-glucopyranoside, *p*-hydroxyphenlacetic acid and β-sitosterols, which also provide its diuretic effect.[22, 26] The bitterness of dandelion can be decreased when subjected to a thermal processing technique such as boiling, but the heat may also degrade and damage the phytochemicals[17, 27] Therefore, it is critical that boiling time effect on phytochemicals need to be established. This research could inform consumers for optimal boiling to reduce bitterness while maintaining optimal health promoting properties.

Dandelion is considered "likely safe" when consumed as food in the form of plant based forms or as a supplementation at the recommended dosage for adults.[28] The FDA, Food and Drug Administration, claims that dandelion is GRAS (Generally Recognized As Safe) as a food additive in the U.S. for consumption at maximum levels of 0.014% for fluid extracts and 0.003% for solid extracts.[28]

*Antioxidant and anti-hepatotoxicity*

Accumulative evidences demonstrated antioxidant inhibition of dandelion leafy greens. However, several studies have shown variation of antioxidant activity due to

species, growing location, maturity, specific parts of dandelion studied (flower, whole leaves or roots), climate and environment, and extraction technique and processing.[13, 29-34] Previous studies have identified phenolic compounds such as chicoric acid and caffeoylquinic acid isomers, flavonoids, in dandelion roots and herb juice from Germany, secondary metabolite terpenoids such as phenolic inositol esters, triterpene acetates and sesquiterpene lactone taraxinic acid b-D-glucopyranosyl ester also from Germany (*Taraxacum officinale* agg.) by methanol and hexane extraction contributing to dandelions' antioxidant potential.[4, 13, 35-44] Dandelion leaf extract has been found to contain chlorogenic acid, caffeic acid, quercetin, phenols, flavonoids, tannins and ascorbic acid which may also aid in contributing to antioxidant activity and antihepatotoxicity to protect and restore antioxidant enzymes.[4, 45-47]

Dandelion varieties possess different physical leaf characteristics that may contribute to its antioxidant potential. Garnet Stem, a unique dandelion variety, has a red midrib and petiole. The red midrib and petiole may contribute to antioxidant activity due to the presence of pigmented flavonoids and anthocyanins. [48] To the best of our knowledge, very little information is available in relation to extraction and identification of anthocyanins of Garnet Stem's red midrib and petiole for extraction and identification of anthocyanins.

*Heart disease and dietary fiber*

Dandelion's dietary fiber may reduce the risk of heart disease by reducing cholesterol, preventing adipogenesis, reducing the production of cellular fat, and increasing the secretion of bile acids to break down cholesterol.[9, 20, 23, 44, 49, 50] Dietary fiber

has been associated with the prevention of heart diseases due to the binding of bile acids.[25] Bile acids are acidic steroids that are synthesized in the liver from chloesterol.[51] After the bile acids conjugate with glycine and taurine, they are secreted into the duodenum and reabsorbed by the ileum and undergo enterohepatic circulation.[25] The synthesis of bile acids helps to break down ingested fatty substances.[19] Foods, such as dandelion leafy greens, can help prevent the reabsorption of bile acids and prevent the stimulation of the plasma and liver cholesterol conversion to other bile acid secondary toxic metabolites.[52]

The role of binding the bile acids with high fibrous leafy green foods helps to increase fecal excretion which can ultimately help lower cholesterol.[52] Bile acid binding, from the consumption of leafy green fractions, seems to be proportional to its dry matter.[51] While very little information is available in relation to bile acid binding capacity of dandelion leafy greens, studies were conducted on bile acid binding with other leafy greens such as kale (9%) and mustard greens(14%).[25] Total dietary fiber according to USDA National Nutrient Database food composition of raw kale is 0.6g, raw mustard greens is 1.8g, and raw dandelion greens is 1.9g per cup.[15] This data suggests that dandelion leafy greens may have a high potential of binding bile acids to reduce cholesterol.

*Chemical composition and phytochemicals from dandelion*

Dandelion chemical composition including: vitamin C, calcium, potassium, total phosphorus, magnesium, oxalic, quinic, malic, ascorbic, citric, and fumaric acids, fiber, nitrates, protein, fat, and vitamin E has been established.[13, 36] Previous studies also identified carotenoids, specifically, lutein, violaxanthin, antheraxanthin, zeaxanthin, neoxanthin, β-carotene, and chlorophyll A and B in dandelion, *Taraxacum officinale* Monivip, from Slovenia.[53] Also, total chlorophyll and carotenoids from dandelion whole leaf and root parts under an environmental stress of increasing concentration of copper contaminated soils over two month time period in Poland was studied.[36]

Due to potential health benefits of consuming dandelion leaves, a more detailed analysis of phytochemicals needs to be conducted based on the different location, period of harvest and/or sample part of dandelion. To best of our knowledge, currently there are no reports on growing location, harvest period and/or plant part effect on antioxidant activity, phytochemicals and bile acid binding capacity of dandelion leafy green varieties. This research outcomes will help consumers to choose the appropriate variety, harvesting time and plant part for consumption of dandelion leaves.

The overall goal of this study was to identify the potential health beneficial compounds and determine the antioxidant activity in dandelion green varieties including whole leaf, leaf blade and midrib/petiole, from different growing locations and times of harvest.

**Research Objectives**

1. To determine the effect of harvest period and location on the levels of phytochemicals, radical scavenging activity and bile acid binding capacity of Catalonga (*Taraxacum officinale*)
2. To determine the recovery of phenolics and radical scavenging activities using different thermal processing techniques on various dandelion varieties
3. To optimize anthocyanins extraction and evaluate the phytochemicals levels in Garnet Stem (*Taraxacum officinale*) harvested from different geographical areas

# CHAPTER II

# EFFECT OF HARVEST PERIOD AND LOCATION, AND THERMAL PROCESSING METHODS ON THE LEVELS OF PHYTOCHEMICALS, BILE ACID BINDING CAPACITY, AND FREE-RADICAL SCAVENGING ACTIVITY OF DANDELION (*TARAXACUM OFFICINALE*) AND CHICORY (*CICHORIUM INTYBUS*)

## Overview

Dandelion (*Taraxacum officinale*) greens have vitamins, minerals, and phytochemicals that may help prevent the onset of human diseases and illnesses. In this study, dandelion variety Catalonga, grown in Texas and New Jersey was separated into whole leaf, leaf blade, and petiole then evaluated for vitamin C, carotenoids, radical-scavenging activity, total phenolic content, bile acid binding capacity, and dietary fiber during early and late harvest periods. Catalonga leaf blade had higher amounts of vitamin C, carotenoids, chlorophyll a and b, radical scavenging activity, and total phenolic content versus the whole leaf and petiole. Catalonga harvested from New Jersey had the highest amount of vitamin C during the late harvest. Catalonga from Texas and New Jersey contained carotenoids and chlorophyll a and b. 2, 2-diphenyl-1picrylhydrazyl (DPPH) scavenging activity and total phenolic content was highest in leaf blade of Catalonga from Texas during the late harvest. Catalonga from Texas had a higher percentage of bound bile acid salts CDCA and DCA. Catalonga from New Jersey contained higher % total dietary fiber during the late harvest. Boiling dandelion leaves

for 15 min or microwaving for 4 min was optimal for highest antioxidant activity. These results indicate that the growing location, harvest period, tissue type, and thermal processing method had different effects on phytochemicals, antioxidant activity, and bile acid binding capacity of Catalonga and Garnet Stem.

**Introduction**

Dandelions (*Taraxacum officinale*) are considered a weed and grow in the lawns of residential areas, along roadsides and highways and in different foraging areas all over the United States of America according to the USDA Natural Resources Conservation Service Plant Database.[54] In their research published in the *Journal of Weed Science*, Wilson et al. studied how to control and decrease the growth of dandelion due its stature as an unwanted perenial.[1, 21, 55] However, the idea that dandelions are weeds focuses on unnecessarily eliminating dandelion greens, which may provide human health benefits.

Dandelions are a part of the Asteraceae family and are used in Native American, Chinese, and European traditional medicine practices.[1, 3] Dandelion is a dark bitter leafy green containing nutrients and bioactive compounds that may help aid human health.[43] Some of these nutrients and bioactive compounds include β-carotene and other carotenoids, dietary fiber, sesquiterpene lactones (taraxinic acid β-D-glucopyranoside, 11, 13-dihydrotaraxinic-acid β-D-glucopyranoside, *p*-hydroxyphenlacetic acid, and β-sitosterols, which give dandelion its bitter taste and degrade when subjected to high temperatures for long periods of time), polyphenolic compounds, phenolics, flavonoids, and coumarins.[5, 22, 24, 53, 56-59]

Other beneficial nutrients in dandelions include vitamins and minerals such as vitamin C, B vitamins, calcium, iron, potassium, manganese, magnesium, and phosphorus.[24, 60] The diuretic effect of these nutrients and phytochemicals can aid in weight loss while the high fiber content improves heart disease, cholesterol absorption, colon cancer, and cardiovascular health.[24, 53, 61] Dandelion also has antioxidant properties that may aid with oxidative stress and inflammation.[38, 62] Despite being perceived as a weed, dandelion greens can be part of a nutritious diet and regular exercise regimen that may help prevent the onset of many diseases and illnesses. Different dandelion components can be used and consumed differently. Dandelion greens can be eaten raw in mixed salads and sautéed with onions and bacon as a great savory side dish. Dandelion root can be juiced with or without other fruits and vegetables as a beverage and brewed with dandelion leaves into hot herbal teas. When dandelion leaves are boiled, the resulting tea can have high antioxidant activity. Dandelion leaf extracted in hot water for 4 hours has been studied as an antioxidant and as an anti-inflammatory agent, to aid as a hepatoprotective from liver damage and liver and kidney function, and with depression.[31, 63] The dandelion leaf hot aqueous extracts contained more polyphenolic compounds, sesquiterpene lactones, terpenoids, flavonoids, etc. compared with dandelion root hot aqueous extracts.[4, 8, 29, 64, 65]

Previous studies have analyzed the phytochemical and health benefits of dandelion root or whole dandelion leaf.[30, 35] In the present study, dandelion variety Catalonga was harvested from two different locations and two different harvesting periods, and was separated into three plant parts: whole leaf, leaf blade, and petiole.

Each sample plant part was evaluated separately for the levels of phytochemicals, antioxidant activity, bile acid binding capacity, and dietary fiber to determine the optimal availability of health beneficial nutrients. Also, to the best of our knowledge, no studies have addressed the exact timing of thermal processing for highest bioavailability of antioxidants in various dandelion and chicory leaf varieties.[4, 8, 22, 29, 64, 65] Efficiency, maximization, and recovery of total phenolic content and radical scavenging activities in dandelion leaves using different thermal processing methods was also investigated in this study.

**Materials and Methods**

*Plant materials*

Catalonga samples were obtained from J&D Produce Inc. harvested in Edinburg, TX early January to late April (**Fig. 1a. and 1b.**) and Vineland, NJ early June to late July (**Fig. 1c. and 1d.**). Catalonga was also obtained from Val Verde Vegetable Co. in McAllen, TX, and Garnet Stem (*Taraxacum officinale*) from J&D Produce Inc. in Edinburg, TX and Vineland, NJ.


**Figure 1. Catalonga dandelions (*Taraxacum officinale*) from Edinburg, TX during early harvest period (a.) and late harvest period (b.), from Vineland, NJ during early harvest period (c.) and late harvest period (d.), and Catalonga plant parts: whole leaf (e.), leaf blade (f.) and petiole (g.) for phytochemical, antioxidant activity and bile acid binding capacity analyses.**

*Chemicals*

Ascorbic acid, gallic acid, β-carotene, lutein, β-cryptoxanthin, violaxanthin, neoxanthin, chlorophyll a, chlorophyll b, enzymes (α-amylase from porcine pancreas, pepsin, pancreatin from porcine pancreas), meta-phosphoric acid (MPA), 2,2-diphenyl-1picrylhydrazyl (DPPH), phosphotungstomolybidic acid (Folin-Ciocalteu reagent), sodium carbonate, methanol, sodium hydroxide, sodium glycodeoxycholate, sodium cholate, sodium deoxycholate, sodium glycochenodeoxycholate, sodium glycocholate, sodium chenodeoxycholate, and mucin from porcine stomach were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nanopure HPLC grade water was purchased from Barnstead/Thermolyne (Dubuque, IA, USA). Tris (2-carboxylethyl) phosphinehydrochloride (TCEP) was purchased from Alfa Aesar (Ward Hill, MA, USA). Orthophosphoric acid 85% (w/w) was purchased from EMD Millipore Corporation (Billerica, MA, USA). All other solvents used as analytical reagents and HPLC grade solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

*Sample preparations*

Catalonga from Texas and New Jersey were harvested at random during their early and late harvest periods. Each sample weighing a total of 300 gm was separated into whole leaf, leaf blade and petiole with a stainless-steel knife (**Fig. 1e.-1g.**) and chopped finely for phytochemical analyses and antioxidant assays. For thermal processing methods and antioxidant assays, Catalonga and Garnet Stem varieties were separated into whole leaves, rinsed with nanopure water, and finely chopped.

*Determination of vitamin C: ascorbic acid, dehydroascorbic acid, and total ascorbic acid*

Vitamin C content was measured by according to our published methods.[66] Chopped fresh samples (2 g) of whole leaf, leaf blade, and petiole were treated with 4 mL of 3% meta-phosphoric acid and extracted by homogenization for 30 s, vortexed for 2 min, and sonicated for 2 h. The samples were centrifuged for 20 min and extracts were passed through 0.45 micron filters and used for ascorbic acid estimation. The above samples (0.5 mL) were treated with 0.5 mL of Tris (2-carboxylethyl) phosphinehydrochloride (28.66 mg TCEP/10 mL of nanopure water) for the reduction of dehydroascorbic acid to ascorbic acid and analyzed by HPLC. Ascorbic acid and total ascorbic acid were quantified on Thermo Scientific HPLC series using Eclipse XDB C-18 (4.6 × 150mm 5 μm pore size) column, with a guard column. Mobile phase 0.03 M phosphoric acid was used with a flow rate of 400 μL/min, and a sample of 10 μL was injected into the HPLC. The absorbance was monitored at 243 nm with a run time of 18 min. The vitamin C was calculated according to a previously described formula.[66]

*Determination of carotenoids and chlorophylls*

Samples (3 g) of whole leaf, leaf blade, and petiole were extracted with 8 mL of acetone, homogenized, vortexed (1 min), sonicated (30 min), and centrifuged (15 min) under dark conditions, and extracts were filtered. The residue was re-extracted twice to recover all the carotenoids, pooled, and stored at -80 °C until HPLC analysis. Waters 1525 HPLC series (Milford, MA, USA) equipped with Waters 717 plus autosampler, Waters YMC C-30, 3-μm column (150 mm × 4.6 mm i.d.) with a guard cartridge (Phenomenex,

Torrance, CA, USA) was used for quantification. Mobile phase (A) methanol and (B) tert-butyl-methyl-ether was used for gradient separation with a flow rate of 1 mL/min. Samples (50 μL) were injected into the HPLC and separated with a runtime of 25 min. All peaks were detected at 450 nm and compounds were identified by comparing retention times and UV spectra to the standards: lutein, β-carotene, β-cryptoxanthin, violaxathin, neoxanthin, chlorophyll a, and chlorophyll b. Quantification of each compound was calculated based on a regression equation and the dilution.

*Determination of DPPH-radical scavenging activity*

DPPH scavenging ability of MeOH triplicate extracted Catalonga Texas and New Jersey whole leaf, leaf blade and petiole samples were measured according to our published method.[67] For each extract, different concentrations (5, 10, 20, 40, 80, and 100 μL) of 0.2 mg/mL of ascorbic acid and 30 μL of extract were pipetted into the wells of a 96-well plate. The total volumes of all the wells were adjusted to 100 μL with MeOH. A total of 180 μL of DPPH (40 mg/L MeOH) was pipetted to all wells and the changes in the absorbance of extracts and standards were measured at 515 nm with a microplate reader (BioTek Instruments, Inc., Winooski, VT) for 30 min. DPPH scavenging activity was expressed as μg/g ascorbic acid equivalents.

*Determination of total phenolics*

Concentrations of total phenolics were determined according to our published paper.[68] MeOH extracts (30 μL) were pipetted into a 96-well plate and the total volume was adjusted to 200 μL with nanopure water. The blank was prepared with 200 μL nanopure water. Volumes (10, 20, 30, 40, 50, 75, and 100 μL) of 0.1 mg/mL gallic acid

were added to all wells and adjusted to 200 µL with nano-pure water. The Folin-Ciocalteu reagent (20 µL of 1 M Folin-Ciocalteu) was added to all wells, incubated for 10 min at 37 °C, then sodium carbonate (40 µL of 0.035 g/mL sodium carbonate) was added to all wells and incubated for 20 min at 37 °C. The absorbance was measured at 760 nm using a microplate reader (BioTek Instruments, Inc., Winooski, VT) after 30 min of incubation at 37 °C. Total phenolics were expressed as µg/g gallic acid equivalents.

*Determination of total insoluble and soluble dietary fiber*

Determination of total insoluble and soluble dietary fiber was conducted using AOAC Official Method 991.43 Total, Soluble, and Insoluble Dietary Fibre in Foods by Medallion Labs (Minneapolis, MN).[69] Sequential enzymatically digested extraction was performed on lyophilized Catalonga whole leaves from Texas and New Jersey harvested during the early and late harvest periods using α-amylase, protease and amyloglucosidase for insoluble dietary fiber. Insoluble dietary fiber from amyloglucosidase solution was filtered, and the residue was washed several times with distilled warm water. A combination of filtrate and warm water was precipitated with 95% EtOH then filtered to obtain soluble dietary fiber. Once soluble dietary fiber was precipitated with EtOH, the residue was filtered, dried and weighed, and total dietary fiber content was determined.

*Determination of bile acid salt binding capacity*

Extraction and quantification of bile acid binding capacity was determined based on the published procedure.[61] Fresh Catalonga leaves (6 g) were chopped, added to 3 mL of nanopure water and subjected to *in vitro* simulation of human digestion including oral

digestion, gastric digestion, and intestinal digestion. Samples were added to 10 mL of α-amylase (3.1 mg in 100 mL of simulated saliva fluid buffer (**Table 1**).[70] Samples were incubated in a shaking water bath (Julabo GmbH SW22, Seelbach, Germany) for 5 min at 37 °C to simulate human oral digestion. The sample pH was adjusted to 2 with 0.1 N HCl and 600 μL of pepsin buffer (200 μg of pepsin/mL 0.1 M HCl) was added. The samples were again incubated for 90 min in shaking water bath at 37 °C to simulate human gastric digestion. The samples were removed and the pH was adjusted to 6.8 with 0.1 N NaOH to stimulate human intestinal digestion, 4 mL of bile acid mixture in 0.05 M phosphate buffer (**Table 2**) and 5 mL of pancreatin (6.25 mg of pancreatin from porcine pancreas/ mL of 50 mM phosphate buffer) were added and incubated for 3 h in the shaking water bath at 37 °C to conclude the human intestinal digestion. The reaction was stopped by inactivating the enzymes at 78 °C, centrifuged for 20 min, and the residue was washed with excess water to remove the adhering bile acids, then the remainder was used for quantification of unbound bile acids.

Unbound bound bile acids were quantified with an Agilent 1200 series HPLC (Foster City, CA, USA) using a Gemini C-18 5-μm column (250 mm × 4.6 mm i.d.) with a guard cartridge (Phenomenex, Torrance, CA, USA). Gradient mobile phase (A) 0.03 mM phosphoric acid and (B) acetonitrile, were used as follows, 10 min 45% A and 55% B, 20 min 10% A and 90%, 25 min 75% A and 25% B, and 35 min 75% A and 25% B with a flow rate of 700 μL/min and 20 μL sample injected with as run time of 32 min. Unbound bile acids were quantified by using regression equations of standard bile acids: sodium glycodeoxycholate (GDCA), sodium cholate (CA), sodium deoxycholate (DCA),

sodium glycochenodeoxycholate (GCDCA), sodium glycocholate (GCA) and sodium chenodeoxycholate (CDCA). The levels of unbound bile acids were calculated by regression equations and dilution factors. The bound bile acids were calculated using the following formula,

Bile acids binding capacity (%) =

$$100 - \left(\frac{mg\ of\ unbound\ bile\ acids\ by\ HPLC * 100}{mg\ of\ bile\ acid\ used\ for\ assay}\right)$$

**Table 1. Simulated salvia fluid in Nanopure water**

| Compound | Concentration (g/L) |
|---|---|
| Sodium chloride | 1.594 |
| Ammonium nitrate | 0.328 |
| Potassium dihydrogen phosphate | 0.636 |
| Potassium chloride | 0.202 |
| Potassium citrate | 0.308 |
| Uric acid sodium salt | 0.021 |
| Urea | 0.198 |
| Lactic acid sodium salt | 0.146 |
| Porcine gastric mucin | 1.000 |

**This data was adopted from Kahlon et al. 2012.[61]**

**Table 2. Bile acid mixture in 0.05 M phosphate buffer**

| Compound | Concentration (mM) |
|---|---|
| Sodium glycocholate (GCA) | 1.24 |
| Sodium cholate (CA) | 12.08 |
| Sodium glycochenodeoxycholate (GCDCA) | 5.14 |
| Sodium glycodeoxycholate (GDCA) | 2.54 |
| Sodium chenodeoxycholate (CDCA) | 5.84 |
| Sodium deoxycholate (DCA) | 13.22 |

**This data was adopted from Kahlon et al. 2012.[61]**

*Determination of soil content and micronutrient analysis*

Soils of the growing area of each Catalonga sample from Texas and New Jersey during the late harvest period were analyzed at the Texas A&M AgriLife Extension Soil, Water, and Forage Testing Laboratory in College Station, TX. Each soil sample was analyzed for pH, conductivity and the amount (mg/kg) of micronutrients.

*Minimally processing of dandelion leafy greens effect on the phenolic compounds*

Boiling, microwaving and hot sonication for three different durations of time was performed to determine phenolics compounds and radical scavenging activities according to previously published protocols.[17, 71]

Boiling: Ten g of freshly chopped dandelion leaves was added to 100 mL pre-heated (100±2 °C) nanopure water in a 250 mL Erlenmeyer flask, boiled for 15 min and the aqueous extract was filtered and measured for total volume. Similarly, 10 g freshly chopped dandelion leaves were processed for 30 min and 120 min to get maximum extraction of phenolics, all extracts were filtered separately and adjusted to 100 mL with

nanopure water. This experiment was performed in triplicate and samples were diluted prior to antioxidant assays.

Microwaving: Ten g of freshly chopped dandelion leaves was added in a 250 mL beaker with 70 mL nanopure water, microwaved to 95 to 100 °C for a total of 2 min and the microwaved extract was removed and filtered. Similarly, freshly chopped dandelion leaves were microwaved for 4 and 6 min to obtain the water extract, filtered separately and adjusted to 100 mL with nanopure water. This experiment was performed in triplicate and diluted prior to antioxidant assays.

Sonication: A third cooking technique, ultra-sonication, was used for a longer duration of time. Ten grams of freshly chopped dandelion leaves was added to 100 mL Nano-pure water in a 250 mL beaker, placed in an ultra-sonicator, covered with a lid, and sonicated for 60 min at 55-65 ºC. The application of energy vibration and heating reaction from the ultra-sonication technique demonstrated an alternative minimally thermal processing method to obtain an aqueous extract.[72] Ultra-sonicated dandelion aqueous extracts were filtered, adjusted to 100 mL with nanopure water, replicated in triplicate, and diluted prior to DPPH scavenging activity and total phenolic antioxidant assays.

*Successive extraction of phenolics using different boiling technique*

This method, extraction of residual phenolics from dandelion leaves by boiling the residue after the first extraction, was determined and evolved from multiple studies.[73-75] Ten g of freshly chopped dandelion was added to 100 mL pre-heated nanopure water (100±2 °C) in a 250 mL Erlenmeyer flask and boiled for 15 min. The

boiled aqueous extract was removed. The residue was extracted for 15 min to get residual compounds. After collecting the 2nd extract, the residue was re-extracted a third time for 90 min. All extracts were filtered, pooled individually, and adjusted to 100 mL with nanopore water. A fourth extract was achieved by combining 10 mL of the three extracts as a recovered dandelion leaf extract. All four extracts were replicated in triplicate, diluted, and analyzed for DPPH scavenging activity and total phenolic antioxidant bioassays.

*Statistical analysis*

Statistical analysis was performed using one-way analysis of variance (ANOVA) with JMP Pro 12.0.1 software. A general linear model was used to test significant differences and means were compared using Students *t*-test at the 5% probability level. Correlations were calculated using Pearson's correlation coefficient ($R$). All results were expressed as means ± SE.

**Results and Discussion**

Previous studies of dandelion have mainly focused on roots and whole leaves; this study examined and compared the phytochemicals, radical scavenging activity, and total phenolics in different tissues harvested in different locations at different times. Dandelion has untapped potential as a healthy leafy green and this study also examined the effects of boiling, microwaving, and sonicating the dandelion leaves on the recovery of phenolic contents and radical scavenging activities.

*Vitamin C: ascorbic acid, dehydroascorbic acid and total ascorbic acid*

Catalonga dandelion samples from Texas and New Jersey were separated into whole leaf, leaf blade, and petiole and each analyzed for vitamin C content: ascorbic acid, total ascorbic acid, and dehydroascorbic acid. Catalonga New Jersey had the highest amount of ascorbic acid and dehydroascorbic acid during the late harvest in the leaf blade (**Table 3**). During the early and late harvest, Catalonga from New Jersey showed significant differences between the whole leaf, leaf blade and petiole for vitamin C content ($p<0.01$). Catalonga grown in Texas had non-detectable amounts of vitamin C in the early harvest; the only detectable amounts were dehydroascorbic acid present in the whole leaf, leaf blade, and petiole during the late harvest. Therefore, samples harvested at different locations and times showed large differences in vitamin C contents.

The reduction of dehydroascorbic acid to ascorbate can be beneficial for brain functionality and prevent brain-related diseases and illnesses.[62] The oxidized form of ascorbate, dehydroascorbic acid, present at high concentrations in the blood serum, can cross the blood-brain barrier into the cells, be reduced to ascorbate, and remain within the cells.[62] Ascorbate can then perform antioxidant functions such as scavenging radical species, recycling α-tocopherol present in the lipid-rich brain cell membranes, and possibly prevent lipid peroxidation.[62] Dehydroascorbic acid can also be reduced and preserve any ascorbate present within the cells at areas of high oxidative stress.[62]

New Jersey leaf had the highest amount of vitamin C content contributed mostly by dehydroascorbic acid, compared with the whole leaf and stem (**Table 3**). Our study

showed a significant difference ($p$<0.05) based on growing location and time of harvest. Previous studies showed that wild dandelion leaves grown in San Luis, Argentina, contained higher vitamin C content compared to Catalonga from Texas and New Jersey, indicating that harvest location plays a vital role in the amount of vitamin C content in dandelion.[14] Also, during post-harvest production, if dandelions are exposed to high temperatures, the amount of vitamin C may decrease.[18] New Jersey Catalonga leaf blade from the late harvest had the best vitamin C levels, based on our findings.

*Carotenoids and chlorophylls*

Carotenoids including violaxanthin, lutein, β-carotene, and chlorophyll a and b were quantified in Catalonga from Texas and New Jersey whole leaf, leaf blade, and petiole samples. The leaf blade samples contained higher amounts of carotenoids and chlorophylls, during the early and late harvest periods, except for β-carotene in the petiole from Texas' early harvest sample (**Table 3**). The Catalonga Texas leaf blade sample had a significant decrease of violaxanthin from early to late harvest (16.53±1.21 to 3.04±0.92 µg/mL) ($p$<0.05). The Catalonga New Jersey leaf blade sample contained significantly higher amounts of β-carotene during the late harvest (46.02±1.65 µg/mL) ($p$<0.05); β-carotene is the main carotenoid responsible for pro-vitamin A activity essential for human nutrition.[76] In the Catalonga from Texas, chlorophyll a and b in the leaf also significantly increased from early to late harvest (392.94±37.22 to 762.64±64.58 and 361.36±33.27 to 709.77±53.21 µg/mL) ($p$<0.05).

We observed significant differences in chlorophylls and carotenoids based on the growing location, early and late harvest periods, and whole leaf, leaf blade and petiole

($p$<0.05).A previous study quantified lutein, violaxanthin, antheraxanthin, zeaxanthin, neoxanthin, β-carotene, α-carotene, chlorophyll a, and chlorophyll b from whole leaf dandelion from Slovenia.[53] In comparison, Catalonga dandelions from Texas and New Jersey contained higher amounts of lutein during the early and late harvest periods in leaf blade and in the whole leaf from New Jersey during the late harvest. Catalonga from Texas and New Jersey also contained higher amounts of violaxanthin in whole leaf and leaf blade samples during Texas' early harvest and New Jersey's late harvest and Texas had higher levels of chlorophyll b in leaf blade during the late harvest. Catalonga var. harvested in San Pietro Vernotico, Italy, had lower levels of lutein and β-carotene comparedto Catalonga whole leaf, leaf blade and petiole from Texas and New Jersey.[77] Other reported compounds neoxanthin, violxanthin, lutein and β-carotene were found present in chicory (*Cichorium intybus*), similar to dandelion, from Sao Paulo, Brazil.[78] In comparison to chicory, Catalonga leaf blade from Texas and New Jersey during the early and late harvest contained higher amounts of lutein and Catalonga leaf blade from New Jersey had higher amounts of β-carotene during the late harvest.

Leafy green vegetables have bountiful amounts of lutein and β-carotene; however, depending on location and the environment, the levels of lutein and β-carotene and other carotenoids and chlorophyll a and b are subject to change.[56, 79] Our data and previously reported research demonstrates that harvest location of dandelion greens plays a role in its levels of carotenoids and chlorophylls.

**Table 3. Vitamin C, carotenoids and chlorophylls in Catalonga dandelion (*Taraxacum officinale*)**

| Compound | Sample | Early Harvest | | Late Harvest | |
|---|---|---|---|---|---|
| | | Texas | New Jersey | Texas | New Jersey |
| TAA | Whole Leaf | ND | 273.2±17.4$^{b}$ | 38.4±1.2$^{b}$ | 302.0±10.7$^{a}$ |
| | Leaf Blade | ND | 399.5±32.8$^{a}$ | 113.6±19.3$^{a}$ | 256.9±12.4$^{b}$ |
| | Petiole | ND | 99.8±7.5$^{c}$ | 25.2±0.3$^{b}$ | 34.1±1.1$^{c}$ |
| AA | Whole Leaf | ND | 171.8±9.4$^{b}$ | ND | 394.3±37.3$^{b}$ |
| | Leaf Blade | ND | 234.4±13.8$^{a}$ | ND | 619.6±16.0$^{a}$ |
| | Petiole | ND | 52.9±0.0$^{c}$ | ND | 55.2±2.4$^{c}$ |
| DHA | Whole Leaf | ND | 101.4±26.4$^{b}$ | 38.4±1.2$^{b}$ | 92.3±31.9$^{b}$ |
| | Leaf Blade | ND | 165.1±7.1$^{a}$ | 113.6±19.3$^{a}$ | 362.6±71.9$^{a}$ |
| | Petiole | ND | 46.8±6.6$^{c}$ | 25.2±0.3$^{b}$ | 21.09±4.2$^{b}$ |
| Violaxanthin | Whole Leaf | 6.75±1.06$^{b}$ | 8.91±1.08$^{b}$ | 0.42±0.17$^{b}$ | 11.66±0.85$^{b}$ |
| | Leaf Blade | 16.53±1.21$^{a}$ | 15.12±0.38$^{a}$ | 3.04±0.92$^{a}$ | 18.30±0.29$^{a}$ |
| | Petiole | 1.12±0.08$^{c}$ | 0.64±0.15$^{c}$ | 0.00±0.04$^{b}$ | 1.26±0.04$^{c}$ |
| Lutein | Whole Leaf | 25.48±2.45$^{b}$ | 49.53±4.25$^{b}$ | 25.99±3.37$^{b}$ | 53.65±4.74$^{b}$ |
| | Leaf Blade | 55.40±4.03$^{a}$ | 78.69±5.37$^{a}$ | 88.71±6.94$^{a}$ | 84.76±4.16$^{a}$ |
| | Petiole | 5.73±0.43$^{c}$ | 5.64±0.13$^{c}$ | 2.52±0.46$^{c}$ | 7.43±0.53$^{c}$ |
| β-Carotene | Whole Leaf | 2.69±0.39$^{b}$ | 14.13±1.01$^{b}$ | 4.27±0.58$^{b}$ | 23.18±0.54$^{b}$ |
| | Leaf Blade | 4.41±0.38$^{a}$ | 17.63±0.84$^{a}$ | 9.67±2.18$^{a}$ | 46.07±1.65$^{a}$ |
| | Petiole | 2.53±0.30$^{b}$ | 3.94±0.13$^{c}$ | 0.77±0.07$^{c}$ | 4.37±0.10$^{c}$ |
| Chlorophyll A | Whole Leaf | 187.08±22.2$^{b}$ | 428.91±43.15$^{b}$ | 226.99±29.67$^{b}$ | 333.21±37.28$^{a}$ |
| | Leaf Blade | 392.94±37.22$^{a}$ | 655.65±47.69$^{a}$ | 762.64±64.58$^{a}$ | 500.04±24.94$^{b}$ |
| | Petiole | 43.95±3.57$^{c}$ | 62.07±2.93$^{c}$ | 33.33±2.35$^{c}$ | 59.96±4.22$^{c}$ |
| Chlorophyll B | Whole Leaf | 172.88±19.67$^{b}$ | 389.79±45.67$^{b}$ | 217.03±24.88$^{b}$ | 326.81±25.55$^{b}$ |
| | Leaf Blade | 361.36±33.27$^{a}$ | 602.26±47.62$^{a}$ | 709.77±53.21$^{a}$ | 508.17±22.54$^{a}$ |
| | Petiole | 44.54±3.37$^{c}$ | 57.49±2.39$^{c}$ | 37.64±3.33$^{c}$ | 58.27±4.94$^{c}$ |

**ND: below level of detectability, AA: ascorbic acid, TAA: total ascorbic acid, DHA: dehydroascorbic acid. Results are presented as mean ± SE and replicated in triplicate. Early Harvest: December 2015 to March 2016 and Late Harvest: April to July 2016. Different letters comparing sample during each harvest period for each compound indicate a significant difference (ANOVA, $p<0.05$).**

*DPPH-radical scavenging activity and total phenolics*

Whole leaf, leaf blade, and petiole MeOH extracts from Catalonga Texas and New Jersey were analyzed for DPPH scavenging activity during both early and late harvest periods (**Fig. 2a.**). DPPH scavenging activities were higher in the leaf blade and whole leaf from New Jersey versus Texas during the early harvest ($p<0.05$). Samples from Texas and New Jersey contained high DPPH scavenging activities during the late harvest but, Texas leaf blade contained the highest DPPH scavenging activity overall during the late harvest. Based on dandelion location and whole leaf, leaf blade, and petiole samples, there was a significant difference during early and late harvest periods ($p<0.0001$).

Total phenolics was determined using Folin-Ciocalteu reagent.[80] Total phenolics levels were similar to DPPH scavenging activities; Catalonga from Texas and New Jersey showed a significant increase in phenolic contents from early to late harvest ($p<0.05$) but, samples from Texas contained the highest levels of phenolics in the leaf blade during the late harvest (**Fig. 2b.**). Based on dandelion location and whole leaf, leaf and petiole tissue, there was a significant difference during each harvest period ($p<0.0001$). A Pearson correlation was conducted to determine the correlation of DPPH scavenging activity and total phenolic antioxidant activity analyses. Catalonga from Texas had an $R$ value of 0.8464 and 0.9743 and New Jersey with 0.9455 and 0.9646 during the early and late harvest resulting in strong positive correlations of the amount of variance between the two antioxidant activity assays.

Depending on dandelion leaf maturity, location, time of harvest and antioxidant extraction techniques may cause of levels of DPPH scavenging activity and total phenolics to increase in the late harvest of Catalonga from Texas and New Jersey.[21, 41, 81, 82] Previously reported DPPH scavenging activities were found to differ based on extraction technique of dandelion flowers, leaves, or roots, from different areas and different times of harvest.[13, 81, 83] Catalonga from Texas and New Jersey during the early and late harvest periods in whole leaf, leaf blade and petiole had higher DPPH scavenging activities compared to dandelion greens subjected to an ultrasound-assisted extraction method, harvested from China.[34] Dandelion root harvested from Ireland, had higher scavenging activity in water extracts, lower activity in ethanol extracts, and higher levels of phenolics in both water and ethanol extracts compared to Catalonga from Texas and New Jersey during the early and late harvest periods.[43] Another study detected high phenolic contents in dandelion leaves from plants exposed to oxidative stress in the presence of heavy metal concentrated soils.[56] Dandelion leaf and root samples from Turkey were extracted with 80% methanol, giving similar total phenolics results compared to Catalonga from Texas and New Jersey during early and late harvest.[31] Our results indicate that the antioxidant potential of dandelion greens is high in the leaf blade during later harvest periods  from Texas. When consumed, these healthy greens can provide optimal flavonoids, phenolic compounds, and other antioxidant compounds.

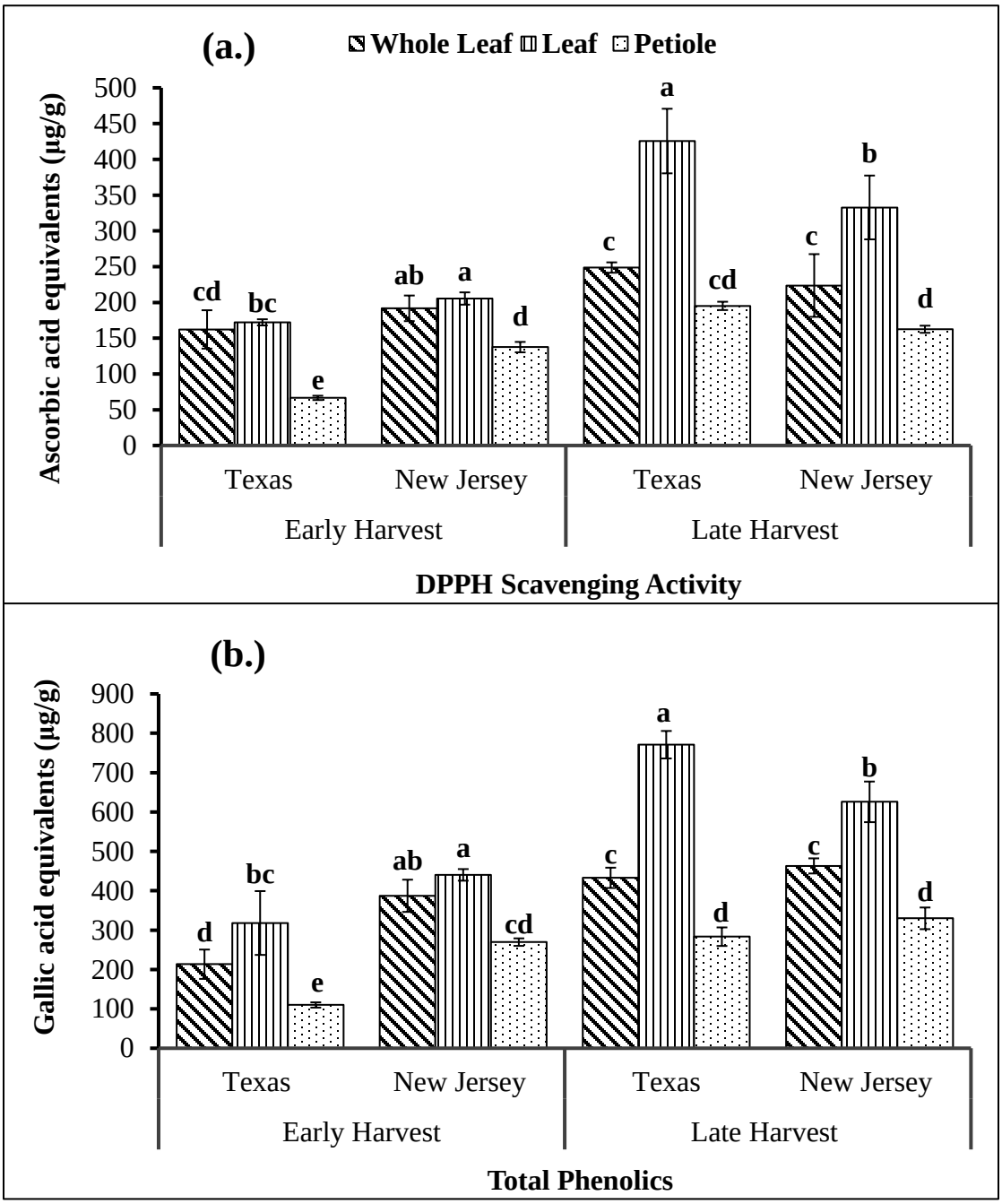


**Figure 2. DPPH scavenging activity (a.) and total phenolics (b.) of Catalonga from Texas and New Jersey during early and late harvest periods. Different letters comparing Texas' and New Jersey's whole leaf, leaf blade and petiole during each harvest period indicate a significant difference (ANOVA, $p<0.05$).**

*Bile acid salt binding capacity and dietary fiber*

Determination of percent bound bile acid salts with Catalonga whole leaves from Texas and New Jersey during the early and late harvest was based on the methods in a previously reported study.[61] Two bile acid salts predominant in the human bile profile are sodium chenodeoxycholate (CDCA) and sodium cholate (CA) and the two bile acid salts that can be toxic to the human body if accumulated at high concentrations are CDCA and sodium deoxycholate (DCA).[84, 85] Catalonga from Texas and New Jersey during the early and late harvest periods showed the highest capacity for binding bile acid salts CDCA and DCA (**Fig. 3.**). Catalonga from Texas had the highest capacity for bound bile acid salts overall during the early and late harvest.

The binding capacities for samples from Texas and New Jersey for all six bile acid salts were significantly different during the early harvest except for CDCA ($p<0.05$) and during the late harvest except for DCA ($p<0.01$). During the early and late harvest, binding capacities to all bile acid salts except for CDCA and DCA were significantly different in samples from Texas vs. New Jersey ($p<0.05$). Also, from early to late harvest, samples from Texas and New Jersey showed significant differences in binding to sodium glycocholate (GCA). Our results demonstrated that dandelion has a high binding capacity for CDCA and DCA bile salts that can be toxic at high levels in the human body.

Another study  determined the different bile acid salt binding capacities of raw and cooked leafy green vegetables.[61] Compared to raw mustard greens, kale, and collard greens, the samples of Catalonga from Texas during the early and late harvest had higher

binding capacities of each bile acid salt.[61] The leafy vegetables subjected to different cooking methods had an increase in bile acid binding capacity, increase in soluble dietary fiber, and decrease of insoluble dietary fiber.[61] This may also be true for dandelion greens if subjected to a cooking method and should be further studied.

Dietary fiber analysis was conducted with freeze dried whole leaves based on the idea that a consumer would eat the whole dandelion leaf to receive the maximum amount of dietary fiber. Dietary fiber was analyzed for Catalonga whole leaves from Texas and New Jersey by Medallion labs (**Table 4**).[69] Catalonga from Texas had a decrease in soluble, insoluble, and total dietary fiber from early to late harvest, unlike Catalonga from New Jersey, which showed no change in soluble fiber but an increase in insoluble and total dietary fiber from early to late harvest. Catalonga from Texas had the highest percentage of total dietary fiber in the early harvest (50.2%) and Catalonga from New Jersey (44.4%) during the late harvest. (**Table 4**).

Previous studies showed that dietary fiber and bile acid salt binding capacity are correlated. Binding capacities of different high-fiber food residues, such as apples, beans and celery concluded that the food residues were able to absorb the bile acid salts and the salts were excreted from the body.[86] Dietary fiber present in specific high-fiber foods can absorb and eliminate bile acid salts from the body.[86] A link between dietary fiber and food also showed that soluble fiber, also known as viscous fiber, binds to the bile acid salts.[87, 88] However, this study disagrees with our results, Catalonga from New Jersey has higher soluble dietary fiber in the early and late harvest period while Catalonga from Texas had higher binding capacity in the early and late harvest.

The soluble dietary fiber slows down digestion, can dissolve and thicken in water to reduce absorption of sugars, and can affect lipid balance by binding to bile acids for elimination from the body.[87] The soluble fiber in dandelion greens is mostly 12-15% inulin β(2→1) fructan gel-like substances.[57, 89] Insoluble dietary fiber absorbs water and passes substances through the colon.[87-89] Based on our results, both Catalonga from Texas and New Jersey should be consumed during early or late harvest for their high fiber content, which will bind bile acids, aid in digestion, and lower cholesterol.[87]

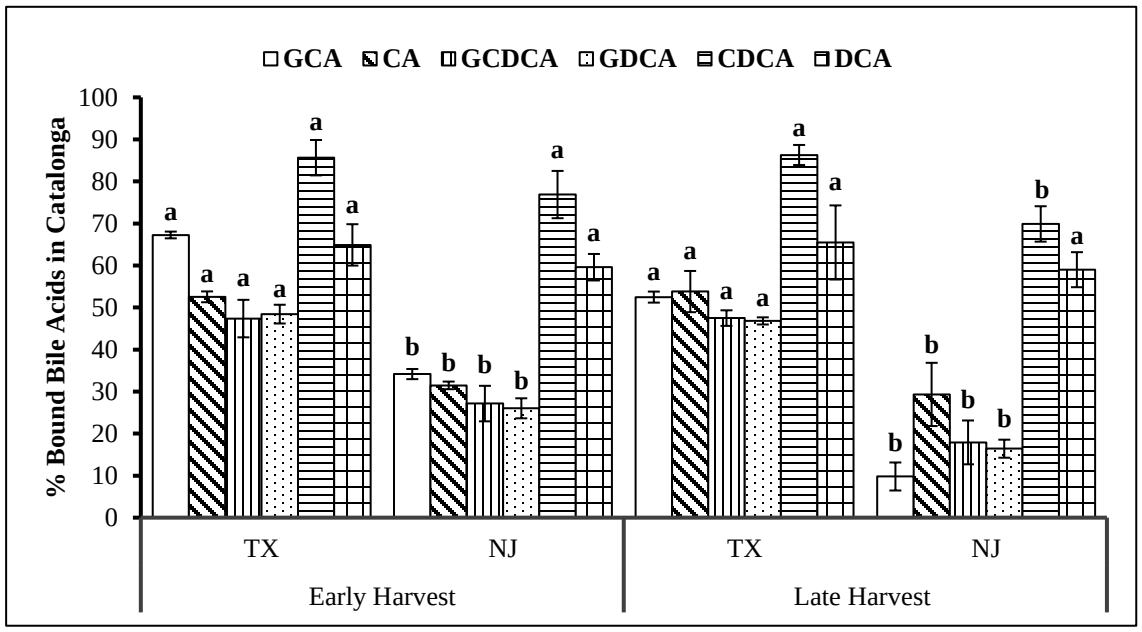


**Figure 3. Percent bound bile acids of Catalonga whole leaf samples from Texas and New Jersey during the early and late harvest periods. Different letters indicate a significant difference between each bile acid and location during early and late harvest (ANOVA $p<0.05$)**

**Table 4. % Dietary fiber in Catalonga (*Taraxacum officinale*) whole leaf**

| | Early Harvest | | Late Harvest | |
|---|---|---|---|---|
| Dietary Fiber | Texas | New Jersey | Texas | New Jersey |
| Soluble Fiber | 8.9 | 9.4 | 8.0 | 9.4 |
| Insoluble Fiber | 41.3 | 31.1 | 34.2 | 35.0 |
| Total Fiber | 50.2 | 40.5 | 42.2 | 44.4 |

**Results were conducted by Medallion Labs (Minneapolis, MN).[69]**

*Soil and micronutrient content*

The soil content was examined during the late harvest of Catalonga from Texas and New Jersey. Soil analyses included: pH, conductivity, and micronutrient presence and content (mg/kg) (**Table 5**). Catalonga from New Jersey had a pH of 6.7 and Catalonga from Texas had a pH of 6.0 but had higher conductivity. The micronutrients present at high concentrations included calcium, phosphorus, potassium, magnesium, and sodium and at lower concentrations, nitrate, sulfur, iron, zinc, manganese, and copper.

Soil contents may affect the amounts of phytochemicals in dandelion greens according to previously reported research. One study demonstrated how soil content played a role in the changing amounts of ascorbic acid present in dandelion leaves over different harvesting periods.[36] Each soil sample for over two months was exposed to copper at increasing concentrations.[36] Within each harvest period, there was a parallel effect; ascorbic acid increased as the concentration of copper increased.[36] However, when the dandelion leaves matured, the ascorbic acid content decreased with the increasing amounts of copper in the soil.[36] Another study tested the effect of increasing concentrations of nitrogen in the soil on the micro- and macro-nutrient profile of chicory (*Cichorium intybus*).[90] A positive correlation was also observed; increasing concentration of nitrogen in the soil increased concentrations of ascorbic acid and other nutrients.[90] Lastly, another study tested the oxidative stress of dandelion by exposing the dandelion to ammonium ion in a nutrient solution, which also increased the total ascorbic acid contents.[56, 91, 92] In our study, the New Jersey soil had 85 and 1.31 mg/kg of

nitrate and copper versus 15 and 0.34 mg/kg of nitrate and copper in Texas possibly resulting in the higher amounts of vitamin C in Catalonga from New Jersey.

**Table 5. Soil and micronutrient content of Catalonga late harvest (ppm: mg/kg)**

| Sample pH, Conductivity and Compounds | Edinburg, TX | Vineland, NJ |
|---|---|---|
| pH | 6.0 | 6.7 |
| Conductivity | 521* | 310 |
| Nitrate-N | 15 | 85 |
| Phosphorus | 116 | 344 |
| Potassium | 239 | 184 |
| Calcium | 1,022 | 1,364 |
| Magnesium | 217 | 106 |
| Sulfur | 153 | 79 |
| Sodium | 207 | 10 |
| Iron | 4.36 | 16.56 |
| Zinc | 1.10 | 2.86 |
| Manganese | 10.39 | 5.55 |
| Copper | 0.34 | 1.31 |

***Units measured in umho/cm**

*DPPH scavenging activity and total phenolics of dandelion leaves extracted by different thermal processing techniques*

Cooking and other thermal processing techniques can affect the antioxidant contents of plant matter. Therefore, we tested our dandelion samples with different thermal processing methods. Garnet Stem dandelion leaves from Texas were chopped up finely, boiled for 15, 30, and 120 min and microwaved for 2, 4, and 6 min separately in

nanopure water, diluted, then analyzed for DPPH scavenging activity and total phenolics (**Fig. 4a.**). As boiling time increased from 15 to 30 min, the DPPH scavenging activity decreased then remained the same from 30 to 120 min. Similarily, as microwaving time increased from 2 to 4 min scavenging activity increased then decreased from 4 to 6 min. DPPH scavenging activity could result from numerous compounds present in the dandelion leaf aqueous extracts, such as secondary metabolites and macronutrients (vitamin C, carotenoids, chlorophyll, flavonoids, phenolics, vitamin E, etc.).[73, 93-96] Observing total phenolic results in **Fig. 5a.**, the trend from 15 to 30 to 120 min. of the boiled and microwaved extracts differ compared with DPPH scavenging activity. In the boiled extracts, the 30 min extract had the highest total phenolics, but this was not statistically different versus the 15 min extract. The 6 min microwaved extract had higher phenolic content versus the 4 min extract, but these were not significantly different. DPPH scavenging activity was highest from boiled and micorwaved Garnet Stem at 15 and 4 min and total phenolic content when boiled from 15 min to 30 min (**Fig. 4a. and 5a.**). These results contradict previously reported findings that showed microwaved aqueous extracts of Catalonga had highest antioxidant activity and total phenols versus boiled Catalonga.[17] Comparing boiled and microwaved treated aqueous extracts, the results showed a significant difference for DPPH scavenging activity ($p$<0.0001) and total phenolics ( $p$<0.01), and both thermal processing techniques had a positive correlation between DPPH scavenging activity and total phenolic content.

Catalonga and Garnet Stem dandelion samples from Texas were subjected to three different heating treatments (**Fig. 4b. and 5b.**). Boiling for 15 min at 100±0.2 ºC,

microwaving for 4 min at 95-100±0.2 ºC, and hot sonication for 60 min at 55 to 65±0.2 ºC. Catalonga aqueous extracts had the highest DPPH scavenging activities for boiling, microwaving, and sonication vs. Garnet Stem aqueous extract which had higher total phenolics when boiled, microwaved, and sonicated. Garnet Stem total phenolic levels were higher than Catalonga due to the anthocyanins present in Garnet Stems red petiole/midrib. Both Catalonga and Garnet Stem had a sigificant differences in DPPH scavenging activity between each thermal processing technique ($p$<0.001 and $p$<0.0001).

Overall, boiled and microwaved aqueous extracts had the highest levels of DPPH scavening activity and total phenolics vs. sonication for Catalonga and Garnet Stem. Correlation between DPPH scavenging activity and total phenolics assays showed a positive correlation. Both Catalonga and Garnet Stem results concluded that boiling or microwaving dandelion leaves for at least 15 min and 4 min was the optimal methods for achieving the highest antioxidant potential for human health. When boiled or microwaved and consumed as a hot tea, these dandelion leafy greens can benefit the body and alleviate oxidative stress.[29, 40]

*Successive extraction and recovery of phenolics using different boiling techniques*

Successive extraction of 5 dandelion leaf varieties was conducted to determine how antioxidants are affected when continously boiled. Also to observe how phenolic compounds are recovered when boiled extracts were combined and compared to the first boiling time of 15 min. Fresh chopped dandelion varieties harvested from different locations were boiled successively for 15, 30, and 120 min and a fourth combination recovery extract were analyzed for DPPH scavenging activity (**Fig. 4c.**) and total

phenolic content (**Fig. 5c.**). The 15 min boiled extract for each variety, except for Catalonga-TX J&D, had the highest DPPH scavenging activity and total phenolic content. DPPH scavenging activity decreased as boiling time increased. All dandelion varieties were significantly different for each 15, 30, 120 min and combination extracts ($p<0.01$). Comparing each boiled time and combination extract for each dandelion variety also showed a significant difference ($p<0.05$) except for Catalonga-TX J&D. DPPH scavenging activity from the three Catalonga varieties was highest in Catalonga from New Jersey when boiled for 15 min. Garnet Stem from Texas had the highest DPPH scavenging activity versus Garnet Stem from New Jersey and the six other varieties for all boiled and combination extracts.

Similar results occurred for total phenolic content with dandelion varieties. Total phenolic content of Catalonga New Jersey was the highest in 15 min and combination extracts compared to the other two Catalonga varieties. Garnet Stem from Texas had higher total phenolic levels compared with Garnet Stem from New Jersey and other varieties for all boiled and combination extracts (**Fig. 5c.**). Each variety had a significant difference between each aqueous extract ($p<0.01$) except for Catalonga Texas and Garnet Stem New Jersey. Comparing all five varieties and recovered extracts, there was a significant difference for total phenolics ($p<0.05$), except between the 120 min extracts not including Garnet Stem-TX J&D. Both DPPH scavenging activity and total phenolics shared a relationship in expression of antioxidant activity resulting in a positive correlation.

These results demonstrated that dandelion varieities freshly boiled contain higher DPPH scavenging activities contrasting with another study, which used dried whole leaves, boiled for a total of 4 hours and had a DPPH scavenging activity of 207±0.84 μg/mL.[45] DPPH scavenging activity was predominately higher in our experiment while total phenolic content results were not. The total phenolics from the 4 hour boiled dried dandelion leaves were higher than all dandelion varieties except for Garnet Stem from Texas.[45]

These results encourage that boiling for 15 min is sufficient for maximum antioxidant benefit from dandelion greens especially from Garnet Stem Texas due to its red midrib/petiole containing anthocyanins contributing to its high antioxidant activity. In a continuation of this study, a sensory analysis should be conducted comparing bitterness of fresh dandelion herbal teas harvested from different locations. It would also be interesting to assess the effects of antioxidant activity from cold-pressed dandelion leaf juice. Today, more cold-pressed juice products are being manufactured for consumers in the market.[97] If cold-pressed dandelion leaf antioxidant potential is just as high or is higher versus boiled dandelion leaf extracts, the results could be a step towards a new way of consuming dandelion.

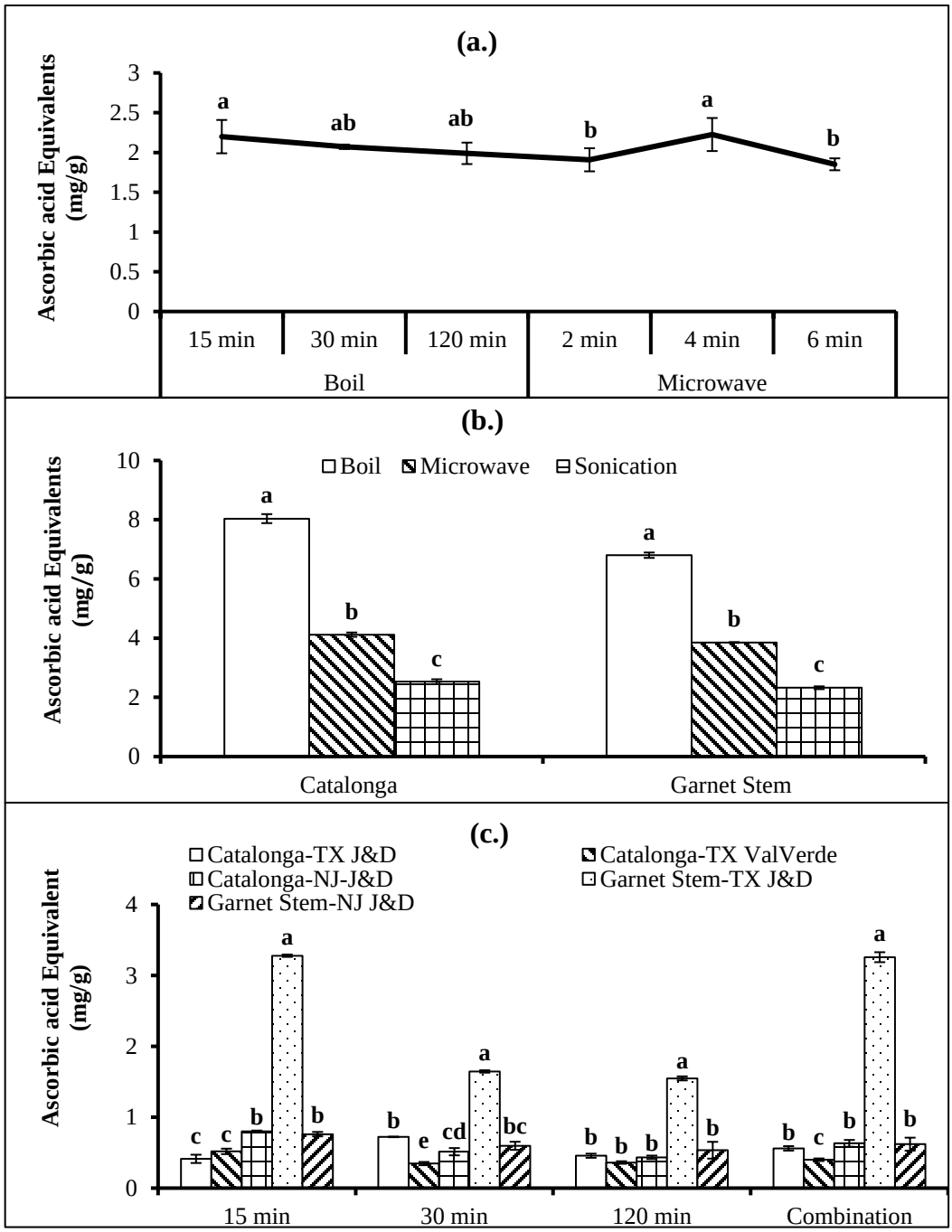


**Figure 4. DPPH scavenging activities of dandelion (*Taraxacum officinale*) whole leaf varieties. (a.) Boiled and microwaved time comparison, (b.) boiled, microwave and sonication comparison, and (c.) successive boiling extraction of phytochemicals. Different alphabets indicate significant differences between each boiled, microwave, and sonicated time and variety (ANOVA $p<0.05$).**

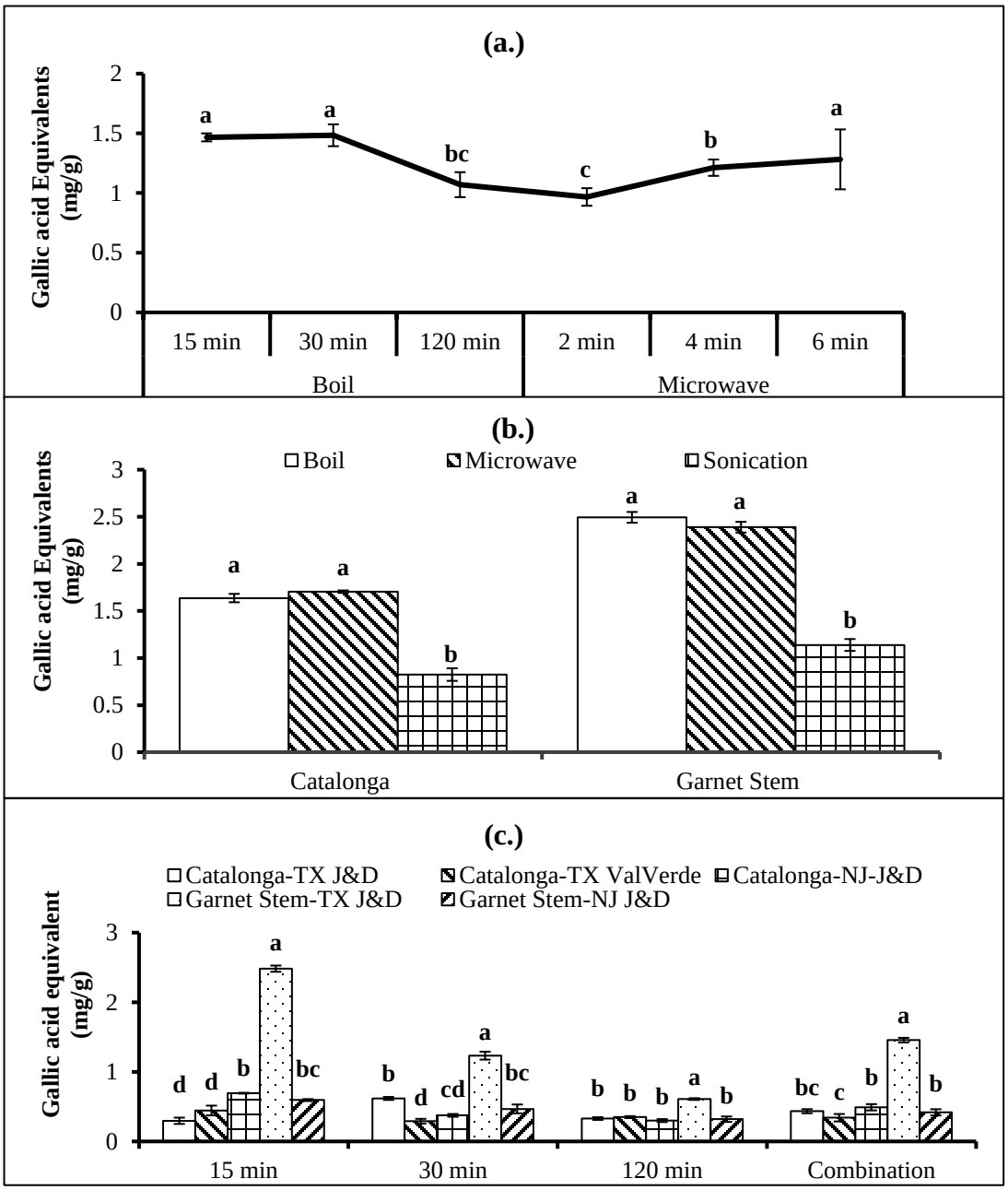


**Figure 5. Total phenolics of dandelion (*Taraxacum officinale*) whole leaf varieties. (a.) Boiled and microwaved time comparison, (b.) boiled, microwave and sonication comparison, and (c.) successive boiling extraction of phytochemicals. Different alphabets indicate significant differences between each boiled, microwave, and sonicated time and variety (ANOVA $p<0.05$).**

## Chapter Summary

Our efforts suggest that the dandelion greens grown in Texas and New Jersey have high potential bioavailability of phytochemicals, antioxidant activity, and dietary fiber to support human health. Higher levels of vitamin C were present in the late harvest of Catalonga leaf blade from New Jersey. Three carotenoids, violaxanthin, lutein, β-carotene and chlorophyll A and B, were quantified in the whole leaf, leaf blade and petiole from Catalonga Texas and New Jersey during their early and late harvest periods. DPPH scavenging activity and total phenolics were highest in the Catalonga leaf blade from Texas during the late harvest. Bile acid binding capacity was higher in Catalonga from Texas. Overall, leaf blade from both Catalonga Texas and New Jersey had the higher content of phytochemicals and antioxidant activities during the late harvest period, compared with the early harvest.

An innovative approach determined what type of heating technqiue to use for a specific time to collect dandelion leaf hot aqueous extracts with the highest potential bioavailabilty of antioxidants. Both boiling and microwaving dandelion leaves are the best techniques to use when cooking dandelion leaves into an herbal tea. However, as boiling time increases, less bitter flavor from the sesquiterpene lactones and antioxidant activity is present in dandelion leaves. Our research was also able to extend our knowledge of how different varieties of dandelion harvested from different locations contain different amounts of DPPH radical scavenging abilities and total phenolic compounds.

There were a few limitations and understandings to still be determined to improve this study. First, the Catalonga Texas and New Jersey samples were not harvest in consecutive months due to lack of growth, weather, and consumer sales. Second, there needs to be better understanding of the ecological systems that play a role in growing the dandelion greens, such as climate, temperature, water, soil and other environmental stresses. Third, an understanding of how macronutrients and micronutrients are taken up from the soil into the dandelion greens correlate with the bioavailability of phytochemicals. Lastly, DPPH is one of many free radicals and it would be interesting to see how these dandelion hot aqueous extracts scavenge other free radicals.

Our results can aid consumers in decisions, such as purchasing mature Catalonga greens and consuming the leaf blade part to ensure higher bioavailability of phytochemicals and antioxidant activities, based on location. Growers can also continue to harvest and increase the availability and yield of dandelion greens based on the phytochemicals, antioxidant activity, and dietary fiber present, which may aid human health similarly to other leafy green vegetables. Fresh dandelion hot teas are a great alternative to consuming raw dandelion leaves but more information about thermally processing fresh dandelion leaves into a shelf-stable product will be required before any industrial implementation could be considered.

# CHAPTER III

# EXTRACTION, IDENTIFICATION, QUANTIFICATION, AND HEALTH-PROMOTING PROPERTIES OF ANTHOCYANINS FROM GARNET STEM DANDELION (*TARAXACUM OFFICINALE)*

## Overview

Dandelion (*Taraxacum officinale*) var. Garnet Stem was harvested from Texas and New Jersey for identification and quantification of phytochemicals, and for measurement of free radical scavenging activity and bile acid binding capacity. The red midvein/petioles were extracted with methanol or ethanol and with or without water in combination with four different acids: formic, hydrochloric, acetic, and citric acid. All 28 different solvent extracts were analyzed by LC-ESI-HR-QTOF-MS to identify four anthocyanins, cyanidin-3-glucoside, cyanidin-3-(6-malonyl)-glucoside (A-1), cyanidin-3-(6-malonyl)-glucoside (A-2), and peonidin-3-(malonyl)-glucoside for the first time. Results from all anthocyanin extracts and antioxidant assays suggest that methanol: water: citric acid (80:19:1) had the highest DPPH scavenging activity and ethanol: water: hydrochloric acid (50:49:1) had the highest total phenolic content. Analysis of phytochemicals in whole leaves, leaf blades and midvein/petioles showed that in the samples from New Jersey, vitamin C and β-carotene were highest in the leaf blades versus whole leaves and midvein/petioles and, in samples from Texas, lutein, violaxanthin, chlorophyll a, and chlorophyll b were highest in leaf blades versus whole leaves and midvein/petioles. The highest bound bile acid salt was sodium chenodeoxycholate (CDCA) and the extracts from plants grown in Texas contained the highest total dietary fiber (44.1%). Results from this

study provide the first report of anthocyanin identification from the midvein and petiole of Garnet Stem dandelions and show that the phytochemicals and nutrients are highest in the leaf blade but may vary in amount depending on harvest location.

**Introduction**

Dandelion (*Taraxacum officinale*) has long been used as a medicinal herb to treat various ailments including dyspepsia, heartburn, spleen and liver complaints, hepatitis, and anorexia.[77, 98] In the United States, dandelion has been considered a weed due to its undesirable perennial growth. Interestingly, despite its status as a weed, dandelion has potential health benefits due to the presence of phenolics, flavonoids, coumarins, terpenoids, sesquiterpene lactones, carotenoids, chlorophylls, dietary fiber, and alkaloids.[4, 21, 22, 42] The reported potential health benefits of dandelion include anti-hepatoxicity, antioxidant activity, diuretic, anti-inflammatory activity, and mitigation of cardiovascular disease.[21, 98]

Dandelion var. Garnet Stem has green leaves with red petioles and leaf midveins, possibly due to the presence of anthocyanins. In leafy vegetables, the red colors mainly result from cyanidin derivatives.[11] For example, the commonly consumed leafy green vegetables red cabbage and red leaf lettuce possess cyanidins.[99] Red cabbage contains higher amounts of cyanidin compared with red leaf lettuce.

Every anthocyanin has the same structural backbone and anthocyanin diversity depends on the number and arrangement of different functional groups. The position of the B-ring and presence of hydroxyl groups at the different positions influences stability and radical scavenging activity.[48] Anthocyanins can become unstable and are strongly

influenced by their chemical structure and the environment including pH, storage temperature and solvents.[48]

For extraction, solvent polarity, pH, and other conditions must be taken into consideration to successfully extract and preserve the water-soluble pigments present. Red leaf pigments need to be extracted under acidic conditions to maintain the red color and anthocyanin stability. Common solvents used for the extraction of anthocyanins include aqueous ethanol or methanol in combination with acid.[48] Depending on the solvent, and the acid, and water ratio, anthocyanins can be extracted and preserved. A previous study reported that methanol with hydrochloric acid effectively extracted anthocyanins from wine grape pomace, compared with ethanol and water with organic acids.[100] In the present study, we aimed to optimize anthocyanin extraction and evaluate antioxidant activities of extracts from the red midvein/petiole tissue of Garnet Stem dandelions, using 28 different solvent compositions. In addition, all the extracts were quantified for the levels of anthocyanins by HPLC, anthocyanins were identified by high-resolution mass spectrometry, and extracts were tested for bile acid binding ability. To examine the effect of environment, we compared the levels of phytochemicals in plants harvested from two different locations.

**Materials and Methods**

*Plant materials*

Dandelion variety Garnet Stem was obtained from J&D Produce Inc., grown in Edinburg, TX during January 2016 and Vineland, NJ during May 2016 (**Fig. 6a. and 6b.**).

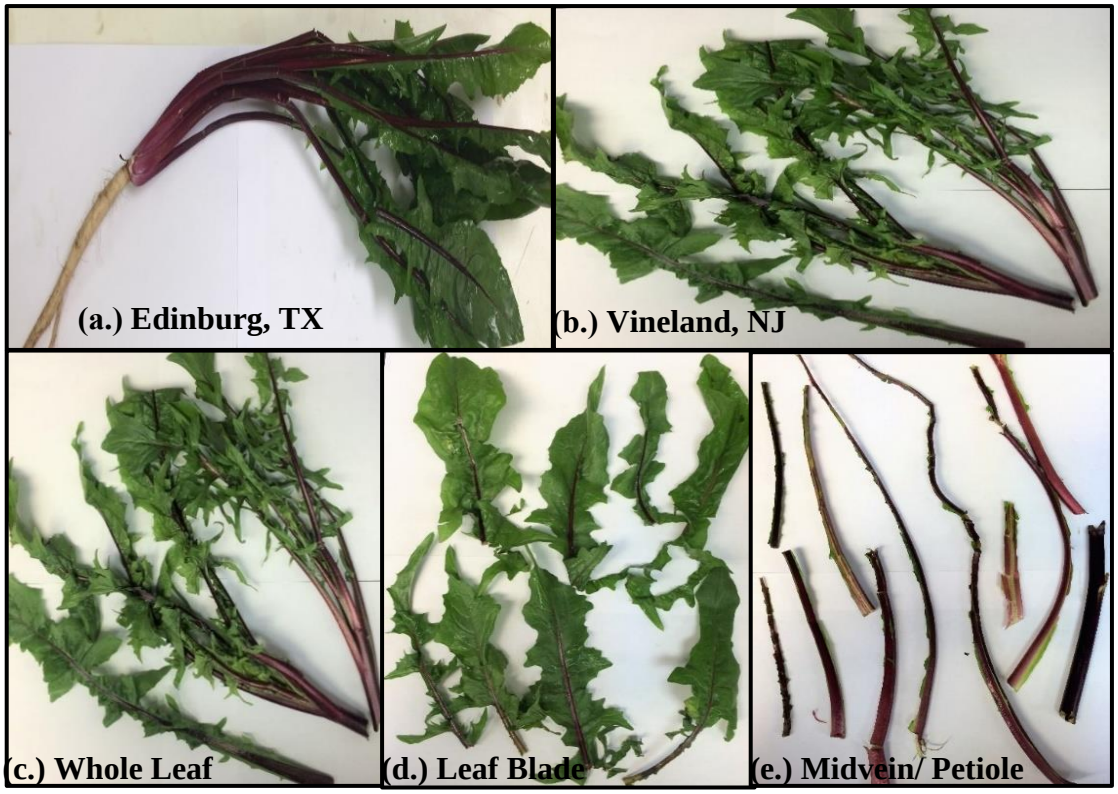


**Figure 6. Garnet Stem dandelion (*Taraxacum officinale*) leafy green samples harvested from different locations (a.) and (b.) and samples processed and used for analysis (c.), (d.), and (e.).**

*Chemicals*

Formic acid, hydrochloric acid, acetic acid, citric acid, methanol, ethanol, phosphoric acid, acetonitrile, tert-butyl methyl ether, meta phosphoric acid (MPA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, Folin-Ciocalteu reagent, and HPLC standards including pelargonidin, ascorbic acid, gallic acid, β-carotene, lutein, β-cryptoxanthin, violaxanthin, neoxanthin, chlorophyll a, and chlorophyll b were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nanopure HPLC grade water was used from

Barnstead/Thermolyne (Dubuque, IA, USA). Tris (2-carboxylethyl) phosphinehydrochloride (TCEP) was purchased from Alfa Aesar (Ward Hill, MA, USA). Ortho phosphoric acid 85% (w/w) HPLC grade was purchased from EMD Millipore Corporation (Billerica, MA, USA).

*Sample preparations*

Midvein/petiole tissue was manually separated with a stainless-steel knife from Garnet Stem dandelion leaf blades from Edinburg, TX (**Fig. 6e.**), chopped finely, and used for optimization of anthocyanin extraction and antioxidant activities. For phytochemical and antioxidant analyses, Garnet Stem plants from Texas and New Jersey were separated into whole leaf (**Fig. 6c.**), leaf blades (**Fig. 6d.**), and midvein/petiole (**Fig. 6e.**) and extracted as described below.

*Optimization of anthocyanin extraction*

Extraction of anthocyanins was optimized with modifications of a procedure from a published paper [101] with 28 solvent compositions containing solvents (water, methanol, ethanol) and different acids (formic, acetic, citric and hydrochloric acid) as mentioned in **Table 6**. Chopped Garnet Stem midvein/petiole tissue (10 g) from Texas was treated with 12 mL of different solvent combinations and extractions were conducted in duplicate. All samples were sonicated for 1 h, in ice cold water (7 °C), vortexed for 2 min and centrifuged at 7826 ×g for 30 min under dark conditions, the supernatant was decanted into new tubes and the residue was re-extracted twice with 6 and 3 mL using respective solvents as mentioned above. All three colored supernatants were pooled, measured to find the total volume, filtered, and stored at -80 °C for HPLC and LC-HR-ESI-QTOF-MS analysis.

**Table 6. Garnet Stem anthocyanin solvent, water and acid extractant combinations**

| Number of solvent combinations | [a]Solvent/Water: Water: Acid Combinations | | | |
|---|---|---|---|---|
| 1 | Water: FA (99:1) | Water: HCl (99:1) | Water: AA (99:1) | Water: CA (99:1) |
| 2 | MeOH: water: FA (80: 19: 1) | MeOH: water: HCl (80: 19: 1) | MeOH: water: AA (80: 19: 1) | MeOH: water: CA (80: 19: 1) |
| 3 | MeOH: water: FA (50: 49: 1) | MeOH: water: HCl (50: 49: 1) | MeOH: water: AA (50: 49: 1) | MeOH: water: CA (50: 49: 1) |
| 4 | MeOH: FA (99: 1) | MeOH: HCl (99: 1) | MeOH: AA (99: 1) | MeOH: CA (99: 1) |
| 5 | EtOH: FA (99: 1) | EtOH: HCl (99: 1) | EtOH: AA (99: 1) | EtOH: CA (99: 1) |
| 6 | EtOH: water: FA (80: 19: 1) | EtOH: water: HCl (80: 19: 1) | EtOH: water: AA (80: 19: 1) | EtOH: water: CA (80: 19: 1) |
| 7 | EtOH: water: FA (50: 49: 1) | EtOH: water: HCl (50: 49: 1) | EtOH: water: AA (50: 49: 1) | EtOH: water: CA (50: 49: 1) |

**MeOH: methanol, EtOH: ethanol, FA: formic acid, HCl: hydrochloric acid, AA: acetic acid and CA: citric acid. [a]Each solvent, water and acid combinations was replicated in duplicate for reproducibility of anthocyanin quantification.**

*Separation and quantification of anthocyanins*

Chromatographic separation of anthocyanins was performed with a Waters 1525 HPLC (Milford, MA, USA) equipped with Waters 717 plus autosampler. Symmetry reverse-phase C-18 and 5-µm (3.9 x 150 mm) column and with a guard cartridge (Phenomenex, Torrance, CA, USA). The gradient mobile phase consisted of (A) 0.03 M phosphoric acid and (B) acetonitrile: water (1:1) with a gradient elution of 70-50% A for 0–2 min, 50-40% A at 2–7 min, 40-70% A at 7–8 min, isocratic for 4 min with a flow rate of 0.5 mL/min. A 20-µL sample was injected at ambient temperatures and the chromatogram was monitored at 520 nm. Quantification of the anthocyanins was achieved

by comparison to a calibration curve. Each anthocyanin was expressed as µg/g of fresh weight of sample.

*LC-ESI-HR-QTOF-MS identification of anthocyanins*

All anthocyanin extracts were analyzed by liquid chromatography electrospray ionization with high resolution quadruple time of flight mass spectrometry (LC-ESI-HR-QTOF-MS). HPLC analyses were carried out using an Agilent 1290 Series Rapid Resolution LC system (Agilent Technologies, Palo Alto, CA, USA), equipped with a vacuum degasser, an autosampler, binary pump, and PDA detector. The column used for the chromatographic separation was a rapid resolution high definition Zorbax Eclipse Plus $C_{18}$ (1.8 µm, 50 mm×2.1 mm) (Agilent Technologies, Palo Alto, CA, USA). In order to obtain separation of the compounds from the dandelion extracts, 0.50 mL/min flow was used at room temperature. The gradient mobile phases used were (A) 0.1% formic acid in LC-MS grade water and (B) 0.1% formic acid in acetonitrile. The following gradient system was applied: 0 min, 5% B; 45 min, 100% B; 55 min, 5% B; and finally, a conditioning cycle of 5 min with the same conditions for the next analysis. The samples (1 µL) were injected and peaks were monitored with a diode array detector between 190 and 640 nm. This LC was coupled with a maXis Impact (Bruker Daltonik, Bremen, Germany) instrument, an orthogonal accelerated TOF mass spectrometer, using an ESI interface. The detection of the compounds of interest was carried out considering a mass range (m/z) 50-1000. External mass spectrometer calibration was performed using a Cole Palmer syringe pump (Vernon Hills, Illinois, USA) directly connected to the interface, equipped with a Hamilton syringe (Reno, Nevada, USA) containing sodium formate (10

mM sodium hydroxide and water: 2-propanol 1:1 (v/v) with 0.2% of formic acid). The calibration solution was injected at the end of each run and all the spectra were calibrated prior to the identification. The accurate mass data for the molecular ions were processed using the software Data Analysis 4.3, which provides the list of possible elemental formulae by using the SmartFormula.

*DPPH scavenging activity of anthocyanin extracts*

DPPH radical scavenging ability of anthocyanins extracts was measured according to our published method.[67] For each anthocyanin midvein/petiole tissue extract, different amounts (5, 10, 20, 40, 80, and 100 μL) of 0.2 mg/mL of ascorbic acid and, 30 μL of extract was pipetted into the wells of a 96-well plate. The total volumes of all the wells were adjusted to 100 μL with MeOH. A total of 180 μL of DPPH (40 mg/L MeOH) was pipetted into all wells and the changes in the absorbance of anthocyanin midvein/petiole extracts and standards were measured at 515 nm with a microplate reader (BioTek Instruments, Inc., Winooski, VT) for 30 min. DPPH scavenging activity was expressed as μg/g ascorbic acid equivalents.

*Total phenolics of anthocyanin extracts*

Concentrations of total phenolics were determined according to our published paper.[68] Anthocyanin petiole/midrib extracts (30 μL) were pipetted into a 96-well plate and the total volume was adjusted to 200 μL with nanopure water. The blank was prepared with 200 μL nanopure water. Different volumes (10, 20, 30, 40, 50, 75 and 100 μL) of 0.1 mg/mL gallic acid were added to all wells and adjusted to 200 μL with nanopure water.

The Folin-Ciocalteu reagent (20 µL of 1 M Folin-Ciocalteu) was added to all wells, incubated for 10 min at 37 °C, then sodium carbonate (40 µL of 0.035 g/mL sodium carbonate) was added to all wells and incubated for 20 min at 37 °C. The absorbance was measured at 760 nm using a microplate reader (BioTek Instruments, Inc., Winooski, VT) after 30 min of incubation at 37 °C. Total phenolics were expressed as µg/g gallic acid equivalents.

*Determination of vitamin C: ascorbic acid, dehydroascorbic acid and total ascorbic acid*

Vitamin C content was measured according to our published protocols.[66] Chopped and ground fresh samples (2 g) of whole leaf, leaf blades and midvein/petiole tissue were treated with 4 mL of 3% meta-phosphoric acid and extracted by homogenization for 30 s, vortexed for 2 min and sonicated for 2 h. The samples were centrifuged for 20 min and extracts were passed through 0.45 micron filters and used for ascorbic acid estimation. The above samples (0.5 mL) were treated with 0.5 mL of Tris (2-carboxylethyl) phosphine hydrochloride (28.66 mg TCEP/10mL of nanopure water) for the reduction of dehydroascorbic acid to ascorbic acid and analyzed for HPLC. Ascorbic acid and total ascorbic acid were quantified on Thermo Scientific HPLC series using Eclipse XDB C-18 (4.6 × 150 mm 5 µm pore size) column, with a guard column. Mobile phase 0.03 M phosphoric acid was used with a flow rate of 0.4 mL/min, and a sample of 10 µL was injected into the HPLC. Absorbance was monitored at 243 nm with a run time of 18 min. The vitamin C was calculated according to a previously described formula.[66]

*Determination of carotenoids and chlorophylls content*

Samples (3 g) of whole leaf, leaf blade, and midvein/petiole tissue were extracted with 8 mL of acetone, homogenized, vortexed (1 min), sonicated (30 min), and centrifuged (15 min) under dark conditions, then the extracts were filtered. The residue was re-extracted twice to recover all the carotenoids, pooled, and stored at -80 °C until HPLC analysis. Waters 1525 HPLC series (Milford, MA, USA) equipped with Waters 717 plus autosampler, Waters YMC C-30, 3-μm column (150 mm × 4.6 mm i.d.) with a guard cartridge (Phenomenex, Torrance, CA, USA) was used for quantification. Mobile phase (A) methanol and (B) tert-butyl-methyl-ether was used for gradient separation with a flow rate of 1 mL/min. Samples (50 μL) were injected into the HPLC and separated with a runtime of 25 min. All peaks were detected at 450 nm and compounds were identified by comparing retention times and UV spectra to the standards: lutein, β-carotene, β-cryptoxanthin, violaxathin, neoxanthin, chlorophyll a, and chlorophyll b. Quantification of each compound was calculated based on a regression equation and the dilution.

*Determination of total, insoluble and soluble dietary fiber*

Determination of total, insoluble, and soluble dietary fiber was conducted using ANNEX G- AOAC Official Method 991.43 Total, Soluble, and Insoluble Dietary Fibre in Foods method by Medallion Labs (Minneapolis, MN).[69]

*Determination of bile acid binding capacity*

Extraction and quantification of bile acid binding capacity was conducted based on the published procedure.[61] Fresh Garnet Stem whole leaves (6 g) were chopped, added

to 3 mL of nanopure water and subjected to *in vitro* simulation of human digestion, including oral digestion, gastric digestion, and intestinal digestion. Samples were added to 10 mL of α-amylase (3.1 mg in 100 mL of simulated saliva fluid buffer (**Table 1**).[70] Samples were incubated in a shaking water bath (Julabo GmbH SW22, Seelbach, Germany) for 5 min at 37 °C to simulate human oral digestion. The sample pH was adjusted to 2 with 0.1 N HCl and 600 μL of pepsin buffer (200 μg of pepsin/mL 0.1 M HCl) was added. The samples were again incubated for 90 min in shaking water bath at 37 °C to simulate human gastric digestion. The samples were removed and the pH was adjusted to 6.8 with 0.1 N NaOH to stimulate human intestinal digestion, 4 mL of bile acid mixture in 0.05 M phosphate buffer (**Table 2**) and 5 mL of pancreatin (6.25 mg of pancreatin from porcine pancreas/mL of 50 mM phosphate buffer) were added and incubated for 3 h in the shaking water bath at 37 °C to conclude the human intestinal digestion. The reaction was stopped by inactivating the enzymes at 78 °C, centrifuged for 20 min, and the residue was washed with excess water to remove the adhering bile acids, then the remainder was used for quantification of unbound bile acids. Unbound bile acids were quantified with an Agilent 1200 series HPLC (Foster City, CA, USA) using a Gemini C-18 5-μm column (250 mm × 4.6 mm i.d.) with a guard cartridge (Phenomenex, Torrance, CA, USA). Gradient mobile phase (A) 0.03 mM phosphoric acid and (B) acetonitrile, were used as follows, 10 min 45% A and 55% B, 20 min 10% A and 90%, 25 min 75% A and 25% B and 35 min 75% A and 25% B with a flow rate of 0.7 mL/min and 20 μL sample injected with a run time of 32 min. Unbound bile acids were quantified by using regression equations of standard bile acids: sodium glycodeoxycholate (GDCA),

sodium cholate (CA), sodium deoxycholate (DCA), sodium glycochenodeoxycholate (GCDCA), sodium glycocholate (GCA), and sodium chenodeoxycholate (CDCA). The levels of unbound bile acids were calculated by regression equations and dilution factors. The bound bile acids were calculated using the following formula:

Bile acid binding capacity (%) =

$$100 - (\frac{mg\ of\ bound\ bile\ acids\ by\ HPLC * 100}{mg\ of\ bile\ acid\ used\ for\ assay})$$

*Statistical analysis*

Statistical analysis was performed using one-way analysis of variance (ANOVA) with JMP Pro 12.0.1 software. A general linear model was used to test significant differences, and means were compared using Student's *t*-test at the 5% probability level. Correlations were calculated using Pearson's correlation coefficient ($R$). The results were expressed as means ± SE.

**Results and Discussion**

*Extraction efficiency of anthocyanins*

To identify the best extraction solvent for the isolation of anthocyanins from Garnet Stem dandelion (**Fig. 6e.**), we used 28 different solvent compositions, which included a wide range of polarities of solvent compositions. All these extracts were analyzed by analytical HPLC for the separation and quantification of anthocyanins. In the dandelion midvein/petiole, four anthocyanins were identified using UPLC-HR-QTOFMS in positive ionization mode. The total ion chromatogram, extracted ion chromatograms, and tandem mass spectra of identified anthocyanins along with broad band collision induced mass spectra (bbCID) are presented in **Fig. 7** UV-Vis spectra show the absorption

at 520 nm, which corresponds to the presence of the characteristic flavylium cation or anthocyanidin nucleus. The tentatively proposed fragmentation patterns of identified anthocyanins are presented in **Fig. 8.** The peak eluted at RT 3.8 min exhibits an accurate mass value at *m/z* 449.1088 $[M]^+$ (mass error 2.2 ppm). In +bbCID spectra, the precursor ion loses one molecule of glucose and gives a prominent base peak at *m/z* 287.0556 $[M-162]^+$ (mass error 2.09 ppm), which corresponds to the cyanidin aglycone moiety. Thus, on the basis of UV-Vis spectra and high resolution mass spectra, the peak at RT 3.8 min was identified as cyanidin-3-glucoside.

Two peaks eluted at RT 4.4 and 4.9 min were considered as isomers, because they had the same mass spectra and UV-Visible spectral data. The peak eluted at RT 4.4 min represents the molecular ion peak at *m/z* 535.1116 $[M]^+$ (mass error 6.4 ppm) along with a characteristic fragment ion peak at *m/z* 287.0568 $[M-162-86]^+$ (mass error 6.3 ppm) which was emerged as a result of the loss of glucose and the malonyl moiety from the molecule. Similarly, the major peak at RT 4.9 shows an accurate mass value at *m/z* 535.1106 $[M]^+$ (mass error 4.5 ppm) and a characteristic aglycone moiety at *m/z* 287.0565 $[M-162-86]^+$ (mass error 5.22 ppm). Similar cyanidin derivatives were also reported in dandelion callus culture.[102] These isomeric cyanidin anthocyanins with malonyl glucosides were also reported in purple maize and are distinguished mainly by their chromatographic elution retention time.[103, 104] On the basis of mass spectral data and published literature, the peak at RT 4.4 and 4.9 were identified as isomer of cyanidin-3-(6˝˝-malonyl)-glucoside and its isomer, which may be possible at β-glyosidic linkage.

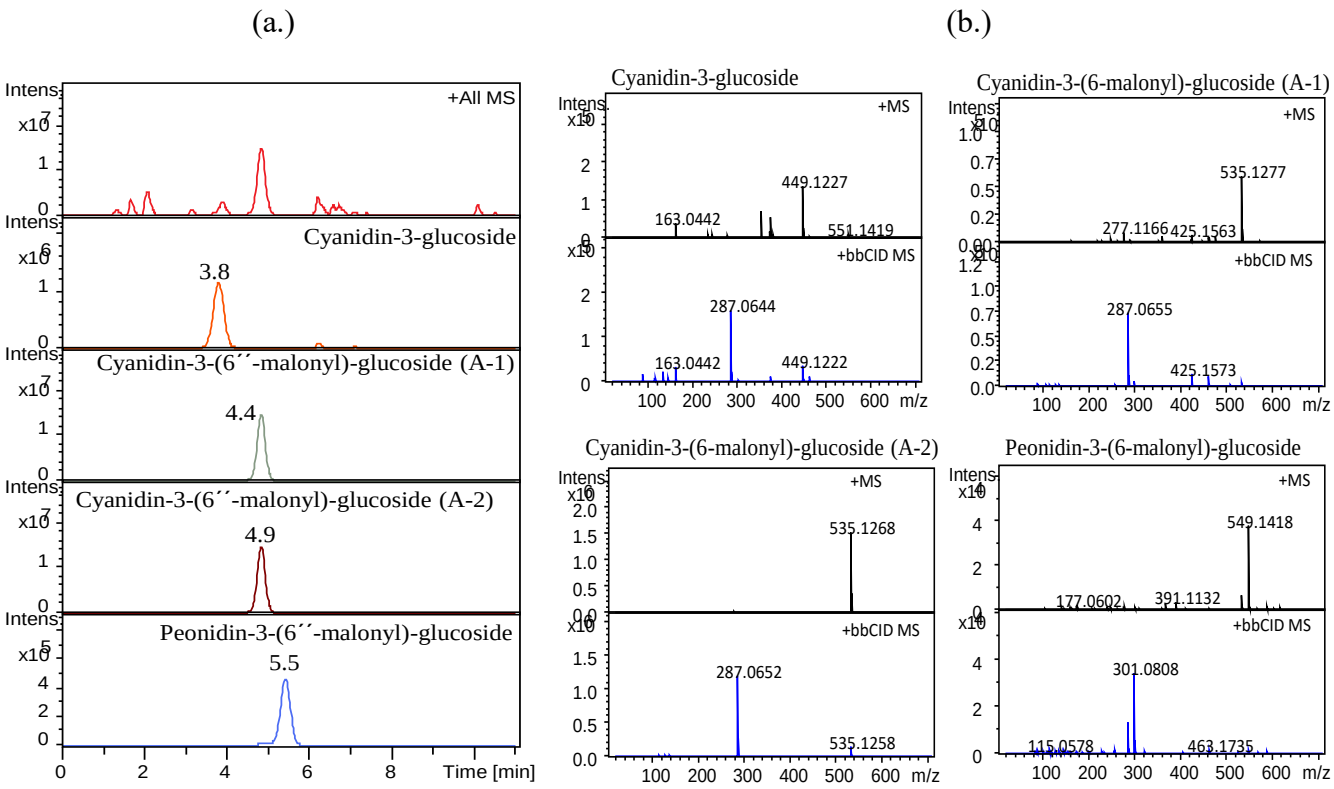


**Figure 7. Extracted ion chromatograms by LC-HR-ESI-QTOF-MS (a.) and Tandem mass spectra of anthocyanins in positive ion mode (b.) identified from the midvein/petiole Garnet Stem dandelions.**

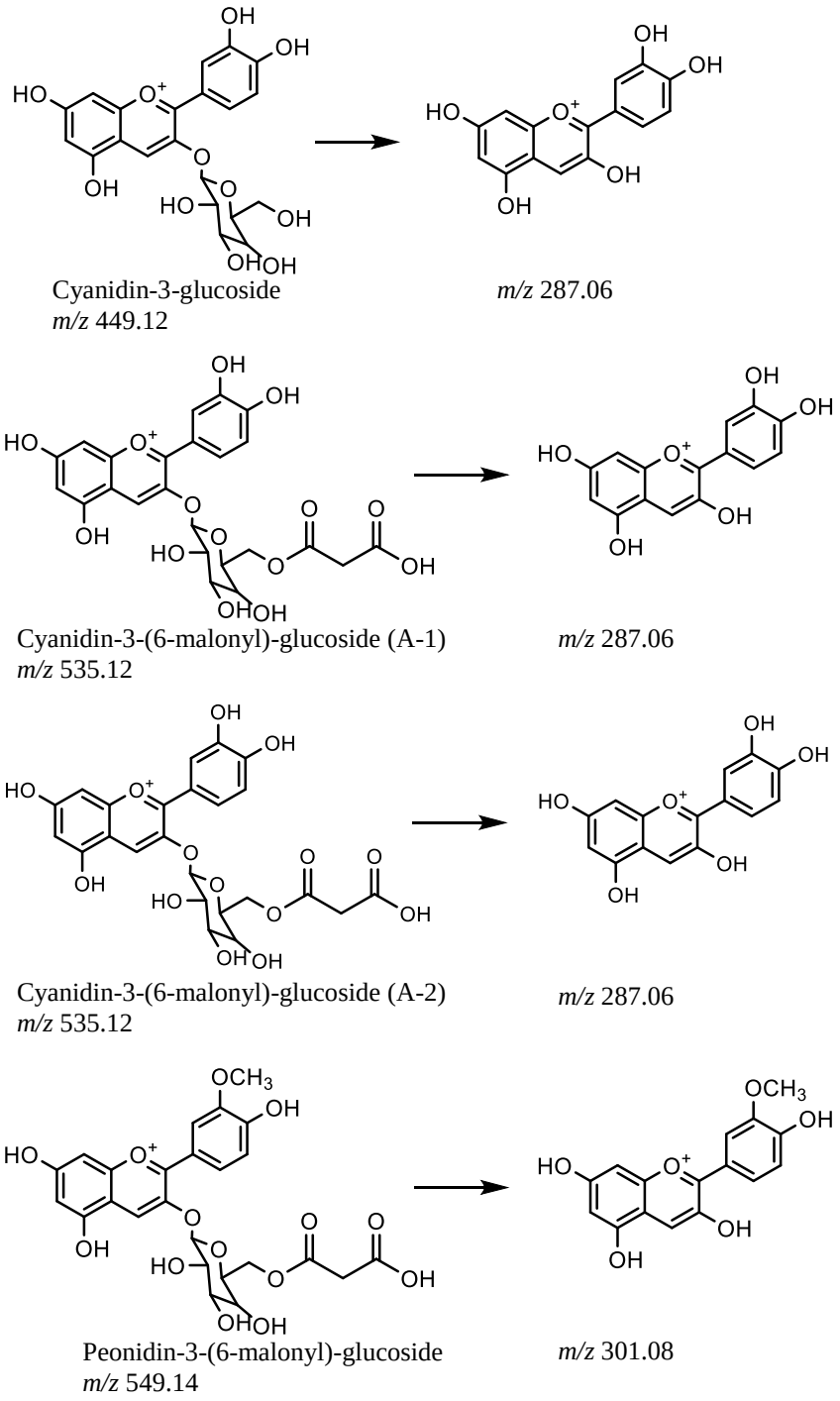


**Figure 8. Proposed fragmentation scheme for anthocyanins (1-4) identified in midveins/petioles of Garnet Stem dandelions.**

Further, the 4th peak eluted at RT 5.5 represents the molecular ion at *m/z* 549.1253 $[M]^+$ (mass error -5.5). It undergoes 3-*O*-glycosidic cleavage to produce a prominent base peak at *m/z* 301.0717 $[M-162-86]^+$ (mass error 3.65 ppm), which corresponds to the peonidin aglycone moiety.

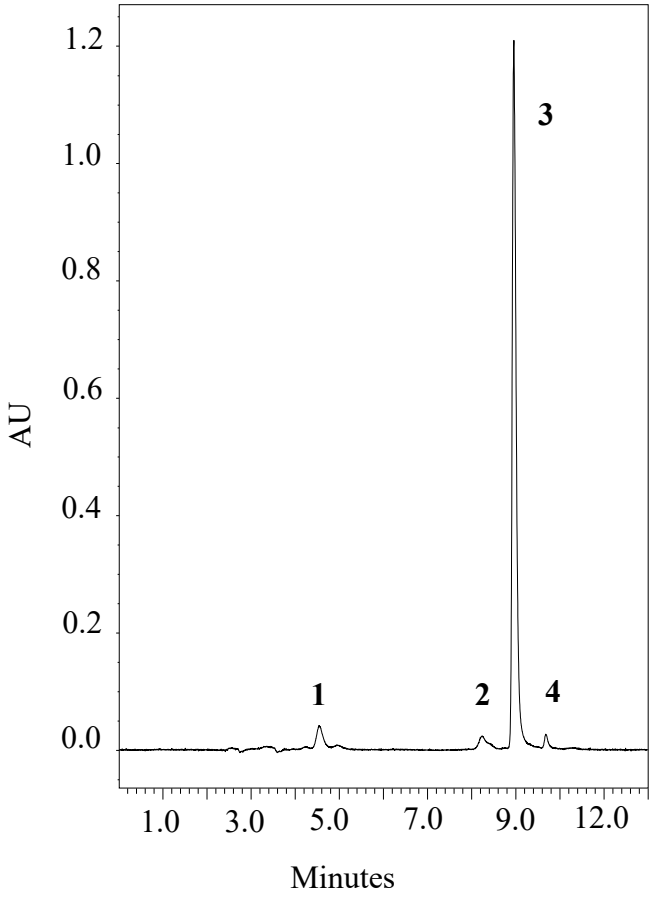


**Figure 9. HPLC Chromatogram of anthocyanins quantified in Garnet red stem (Taraxcum officinale) from Edinburg, TX. Peak 1- Cyanidin-3-glucoside, Peak 2- Cyanidin-3-(6-malonyl)-glucoside A-1, Peak 3- Cyanidin-3-(6-malonyl)-glucoside A-2 and Peak 4- Peonidin-3-(malonyl)-glucoside.**

On the basis of the fragmentation pattern and accurate mass, the present peak was identified as peonidin-3-(6´´-malonyl)-glucoside. These four anthocyanin peaks were detected at 520 nm (**Fig. 9.**) and those were identified as cyanidin-3-glucoside, cyanidin-3-(6-malonyl)-glucoside A-1, cyanidin-3-(6-malonyl)-glucoside A-2 and peonidin-3-(malonyl)-glucoside (**Fig. 7.**).

The levels of anthocyanin varied significiantly in the different solvent compositions (**Table 7**). The level of cyanidin-3-glucoside was significantly higher (4.96±0.26 μg/g) in MeOH: acetic acid (99:1). Cyanidin-3-(6-malonyl)-glucoside A-1 and peonidin-3-(malonyl)-glucoside were found to be maximal (4.43±0.09 and 3.78±0.05 μg/g) in MeOH: water: citric acid (CA) (80:19:1) respectively. The major cyanidin-3-(6-malonyl)-glucoside A-2 was found to be highest (35.78±1.95 μg/g) in MeOH: formic acid (FA) (99:1). Based on a 2-way ANOVA, an overall significant difference occurred between all MeOH, EtOH, and water extraction solvents versus each concentration of acid ($p$<0.01) in quantifying cyanidin-3-glucoside and cyanidin-3-(6-malonyl)-glucoside A-2. Comparing FA, hydrochloric acid (HCl), AA and CA in each extraction solvent (1-7) (**Table 6**), there was a significant difference ($p$<0.05) except in solvent: water: acid combinations 3, 4, 6, and 7 for each compound.

These results suggest that there was a significant difference ($p$<0.05) using the seven different extractant solvents combinations with each acid but the best extraction solvent for Garnet Stem was found to be MeOH: FA (99:1) or MeOH: water: FA (80:19:1) (**Table 7**). However, most of the reported studies have used MeOH: HCl to extract anthocyanins.[101, 102, 105, 106] This may be due to the nature of the plant matrix, moisture

content, and polarity of the anthocyanins present in each crop. All acids used in this study were natural except HCl. Anthocyanins extracted with organic acids has a great potential to use as coloring agents in food formulations.[48, 99]

The bioavailability of anthocyanins for their potential roles as antioxidants has been studied.[107] Anthocyanins are metabolized forming multiple derivatives in the gastrointestinal tract, mostly being absorbed in the small intestine. Some small unmetabolized parent compounds from anthocyanins have been identified in circulation systems as well as in urine samples from human pharmacokinetics and animal studies. Low bioavailability of certain anthocyanins may be due to the attachmnet of di- or trisaccharides to the parent anthocyanin moiety, but recent studies have discovered that lower molecular weight phenolic and aromatic ring-fission catabolites form from anthocyanins and are much more bioavailable. The primary bioavailable products of anthocyanin upon consumption are the catabolites, which may be responsible for the reported bioactivity of anthocyanins.[107]

**Table 7. Levels of anthocyanins quantified midveins/petioles by HPLC (µg/g)***

| Compound | Extraction Solvent | FA | HCl | AA | CA |
|---|---|---|---|---|---|
| Cyanidin-3-glucoside | Water: acid (99:1) | 3.5±0.1$^{ab}$ | 3.1±0.0$^{c}$ | 3.2±0.0$^{bc}$ | 3.7±0.1$^{a}$ |
| | MeOH: water: acid (80:19:1) | 4.4±0.0$^{a}$ | 3.7±0.2$^{b}$ | 4.3±0.3$^{b}$ | 4.7±0.1$^{a}$ |
| | MeOH: water: acid (50: 49:1) | 4.3±0.1$^{a}$ | 4.0±0.1$^{a}$ | 4.1±0.1$^{a}$ | 4.3±0.2$^{a}$ |
| | MeOH: acid (99: 1) | 4.5±0.1$^{ab}$ | 4.0±0.2$^{b}$ | 4.9±0.2$^{a}$ | 4.3±0.2$^{ab}$ |
| | EtOH: acid (99:1) | 3.9±0.1$^{a}$ | 3.3±0.1$^{b}$ | 3.4±0.2$^{b}$ | 3.0±0.0$^{b}$ |
| | EtOH: water: acid (80: 19: 1) | 4.2±0.1$^{a}$ | 3.7±0.3$^{ab}$ | 3.4±0.1$^{b}$ | 3.3±0.1$^{b}$ |
| | EtOH: water: acid (50: 49: 1) | 3.9±0.0$^{a}$ | 3.4±0.0$^{a}$ | 3.1±0.1$^{a}$ | 3.7±0.2$^{a}$ |
| Cyanidin-3-(6-malonyl)-glucoside A-1 | Water: acid (99:1) | 3.3±0.1$^{a}$ | 3.0±0.0$^{ab}$ | 2.8±0.0$^{b}$ | 3.2±0.0$^{a}$ |
| | MeOH: water: acid (80:19:1) | 3.9±0.1$^{ab}$ | 3.4±0.2$^{c}$ | 3.5±0.1$^{bc}$ | 4.4±0.0$^{a}$ |
| | MeOH: water: acid (50: 49:1) | 3.7±0.1$^{a}$ | 3.2±0.2$^{c}$ | 3.3±0.1$^{bc}$ | 3.6±0.3$^{ab}$ |
| | MeOH: acid (99: 1) | 4.1±0.1$^{a}$ | 3.6±0.1$^{b}$ | 4.1±0.0$^{a}$ | 3.4±0.0$^{b}$ |
| | EtOH: acid (99:1) | 4.0±0.1$^{a}$ | 3.3±0.0$^{b}$ | 3.2±0.2$^{b}$ | 2.8±0.0$^{c}$ |
| | EtOH: water: acid (80: 19: 1) | 4.0±0.0$^{a}$ | 3.3±0.2$^{b}$ | 3.0±0.1$^{b}$ | 3.0±0.0$^{b}$ |
| | EtOH: water: acid (50: 49: 1) | 3.7±0.1$^{a}$ | 3.1±0.0$^{a}$ | 3.0±0.0$^{a}$ | 3.3±0.3$^{a}$ |
| Cyanidin-3-(6-malonyl)-glucoside A-2 | Water: acid (99:1) | 14.1±1.5$^{a}$ | 7.9±0.3$^{d}$ | 9.6±0.2$^{c}$ | 11.6±0.6$^{b}$ |
| | MeOH: water: acid (80:19:1) | 32.9±1.6$^{a}$ | 21.4±1.9$^{c}$ | 27.9±1.5$^{b}$ | 33.4±1.8$^{a}$ |
| | MeOH: water: acid (50: 49:1) | 26.5±1.3$^{a}$ | 21.1±2.0$^{b}$ | 23.7±1.6$^{ab}$ | 23.6±2.1$^{ab}$ |
| | MeOH: acid (99: 1) | 35.7±1.9$^{a}$ | 27.2±1.3$^{c}$ | 33.7±1.8$^{ab}$ | 30.3±2.4$^{bc}$ |
| | EtOH: acid (99:1) | 29.2±0.6$^{a}$ | 22.7±0.7$^{b}$ | 16.7±0.4$^{c}$ | 11.2±0.6$^{d}$ |
| | EtOH: water: acid (80: 19: 1) | 29.9±0.9$^{a}$ | 19.1±1.7$^{b}$ | 15.6±1.8$^{bc}$ | 13.2±1.3$^{c}$ |
| | EtOH: water: acid (50: 49: 1) | 23.2±0.9$^{a}$ | 16.9±0.1$^{ab}$ | 14.6±0.4$^{b}$ | 15.7±0.3$^{b}$ |
| Peonidin-3-(malonyl)-glucoside | Water: acid (99:1) | 3.2±0.1$^{a}$ | 2.9±0.0$^{b}$ | 2.9±0.0$^{b}$ | 3.2±0.1$^{a}$ |
| | MeOH: water: acid (80:19:1) | 3.5±0.1$^{a}$ | 3.0±0.1$^{b}$ | 3.1±0.1$^{b}$ | 3.7±0.0$^{a}$ |
| | MeOH: water: acid (50: 49:1) | 3.5±0.1$^{a}$ | 2.9±0.0$^{c}$ | 3.2±0.0$^{b}$ | 3.4±0.1$^{a}$ |
| | MeOH: acid (99: 1) | 3.6±0.0$^{a}$ | 3.2±0.0$^{b}$ | 3.3±0.1$^{ab}$ | 3.4±0.1$^{ab}$ |
| | EtOH: acid (99:1) | 3.5±0.1$^{a}$ | 2.8±0.0$^{c}$ | 3.1±0.0$^{b}$ | 2.8±0.0$^{c}$ |
| | EtOH: water: acid (80: 19: 1) | 3.5±0.0$^{a}$ | 3.2±0.0$^{b}$ | 3.0±0.1$^{b}$ | 3.1±0.0$^{b}$ |
| | EtOH: water: acid (50: 49: 1) | 3.3±0.0$^{a}$ | 2.9±0.0$^{a}$ | 2.8±0.1$^{a}$ | 3.2±0.0$^{a}$ |

***: All results were expressed as relative to pelargonidin**

**FA: formic acid, HCl: hydrochloric acid, AA: acetic acid, CA: citric acid**

**All results are presented as mean ± SE and repeated in triplicates.**

**Different alphabets within the column indicates the significant differences between extraction of anthocyanins using particular acid (ANOVA, $p$<0.05)**

*DPPH scavenging activity and total phenolics of anthocyanin extracts*

All anthocyanin extracts from different solvent compositions were analyzed for DPPH free radical scavenging activity and total phenolics (**Table 8**). The correlation between DPPH free radical scavenging activity and total phenolics in various solvent compositions were analyzed by ANOVA. The FA and CA in each of its seven solvent combinations demonstrated a significant difference ($p<0.01$). Comparing FA, HCl, AA, and CA in each extraction solvent (1-7) (**Table 6**), there was a significant difference ($p<0.05$) except in solvent: water: acid combinations 1, 6, and 7 for DPPH scavenging activity. Overall, MeOH: water: CA (80:19:1) extract was found to have the highest DPPH scavenging activity (575.4±24.4 μg/mL). HCl and AA in each of their seven solvent ratios, showed no significant difference in scavenging DPPH.

The DPPH scavenging activity of each solvent: water: acid combination, in descending order was: FA, AA and CA, and HCl. These results indicated that solvent/water/FA combinations had the highest DPPH scavenging activity and FA was the best acid to preserve the anthocyanins and other radical scavenging compounds from the red midvein/petiole of Garnet Stems dandelions. Results of solvent and water combinations suggest that MeOH: water: acid (80:19:1) and MeOH: acid (99:1) are the best solvent and water extraction combinations to stabilize and preserve the anthocyanins and other phenolics present in the samples.

DPPH free radical scavenging activity was influenced by the solvent, water, and acid combinations. Anthocyanins are susceptible to oxidation because they are water soluble compounds. MeOH: water: CA (80: 19: 1) and MeOH: acid (99: 1) with FA or

AA demonstrated the best ability for stabilizing anthocyanins and other phenolic compounds. Lastly, DPPH scavenging activity of the anthocyanin extracts may have also been influenced by the presence of other compounds such as vitamin C, tocopherols, carotenoids, etc in obtaining various DPPH scavenging activities.[93, 108]

Based on ANOVA, significant differences in phenolic content was observed between all 7 solvent extract combinations for FA, HCl, AA, and CA ($p$<0.01) (**Table 8)**. Highest total phenolic content (939.8±10.9 μg/mL) was achieved with EtOH: water: HCl (50: 49: 1), indicating its ability to preserve the anthocyanins and other phenolics present for quantification of gallic acid equivalents (**Table 8**). A significant difference occurred for CA ($p$<0.0001), FA ($p$<0.001), AA ($p$<0.01), and HCl ($p$<0.05) in each of their seven solvent and water combinations. Comparing FA, HCl, AA, and CA in each extraction solvent (1-7) (**Table 6**), there was a significant difference ($p$<0.05) except in solvent: water: acid combinations 1, 4, and 7 for total phenolic content. The total phenolics could vary if the best extraction solvent: water: acid combination stabilized phenolic compounds. Total phenolic contents were achieved highest in FA, followed by HCl and CA and AA, and observing the seven solvent: water: acid combinations total phenolic content sums and ANOVA, EtOH: water: acid (80: 19: 1 or 50: 49: 1) was the best to extract Garnet Stems red midvein/petioles for phenolic compound contents.

The correlation between DPPH scavenging activity and total phenolics with all anthocyanin extracts were found to be strong positive correlation between DPPH scavenging activity and total phenolics for all MeOH, EtOH, and water compositions. We found excellent correlations coefficients ($R^2$) for FA, HCl, AA, and CA were 0.9213,

0.8729, 0.8293, and 0.943 (**Fig. 10.**). These positive corrections could suggest that DPPH scavenging activity is directly influenced by the phenolic compounds present in each extract.

**Table 8. DPPH free scavenging activity (µg/g of ascorbic acid equivalents) and total phenolics (µg/g of gallic acid equivalents) of extracted Garnet Stem anthocyanins**

| Analysis | Extraction Solvent | FA | HCl | AA | CA |
|---|---|---|---|---|---|
| DPPH Scavenging Activity | Water: acid (99:1) | 323.4±56.3$^{a}$ | 289.4±9.7$^{a}$ | 379.1±20.2$^{a}$ | 370.0±17.2$^{a}$ |
| | MeOH: water: acid (80:19:1) | 537.1±32.2$^{a}$ | 430.5±30.7$^{b}$ | 548.7±26.9$^{a}$ | 575.4±24.4$^{a}$ |
| | MeOH: water: acid (50: 49:1) | 502.8±27.7$^{a}$ | 415.9±24.7$^{b}$ | 533.9±22.9$^{a}$ | 512.2±15.5$^{a}$ |
| | MeOH: acid (99: 1) | 551.0±12.7$^{a}$ | 432.6±32.5$^{b}$ | 550.1±36.4$^{a}$ | 535.7±24.0$^{a}$ |
| | EtOH: acid (99:1) | 513.1±12.7$^{a}$ | 388.2±32.4$^{c}$ | 499.9±19.7$^{ab}$ | 458.2±13.0$^{b}$ |
| | EtOH: water: acid (80: 19: 1) | 544.4±17.7$^{a}$ | 411.7±41.5$^{a}$ | 518.7±53.8$^{a}$ | 477.7±14.2$^{a}$ |
| | EtOH: water: acid (50: 49: 1) | 486.5±30.4$^{a}$ | 451.3±83.0$^{a}$ | 464.7±20.4$^{a}$ | 491.3±32.0$^{a}$ |
| Total Phenolics | Water: acid (99:1) | 554.4±5.3$^{a}$ | 385.3±4.8$^{b}$ | 488.4±10.1$^{a}$ | 419.7±26.1$^{ab}$ |
| | MeOH: water: acid (80:19:1) | 836.6±39.2$^{a}$ | 668.5±48.1$^{b}$ | 688.4±0.7$^{b}$ | 802.0±13.7$^{a}$ |
| | MeOH: water: acid (50: 49:1) | 745.5±15.1$^{a}$ | 647.2±11.0$^{b}$ | 636.5±24.8$^{b}$ | 664.2±26.3$^{b}$ |
| | MeOH: acid (99: 1) | 837.2±32.2$^{a}$ | 709.3±22.9$^{b}$ | 717.4±22.4$^{b}$ | 745.2±49.4$^{ab}$ |
| | EtOH: acid (99:1) | 725.7±22.0$^{a}$ | 656.7±23.6$^{ab}$ | 541.4±42.9$^{c}$ | 624.1±3.08$^{b}$ |
| | EtOH: water: acid (80: 19: 1) | 850.3±19.4$^{a}$ | 711.5±19.8$^{b}$ | 626.3±20.0$^{c}$ | 641.6±26.3$^{bc}$ |
| | EtOH: water: acid (50: 49: 1) | 780.4±34.7$^{a}$ | 939.8±10.9$^{a}$ | 576.5±7.0$^{a}$ | 692.0±9.6$^{a}$ |

**All results are presented as mean ± SE and repeated in triplicates**
**Different alphabets comparing each 1% acid in each extraction solvent indicate a significant difference (ANOVA, $p<0.05$)**

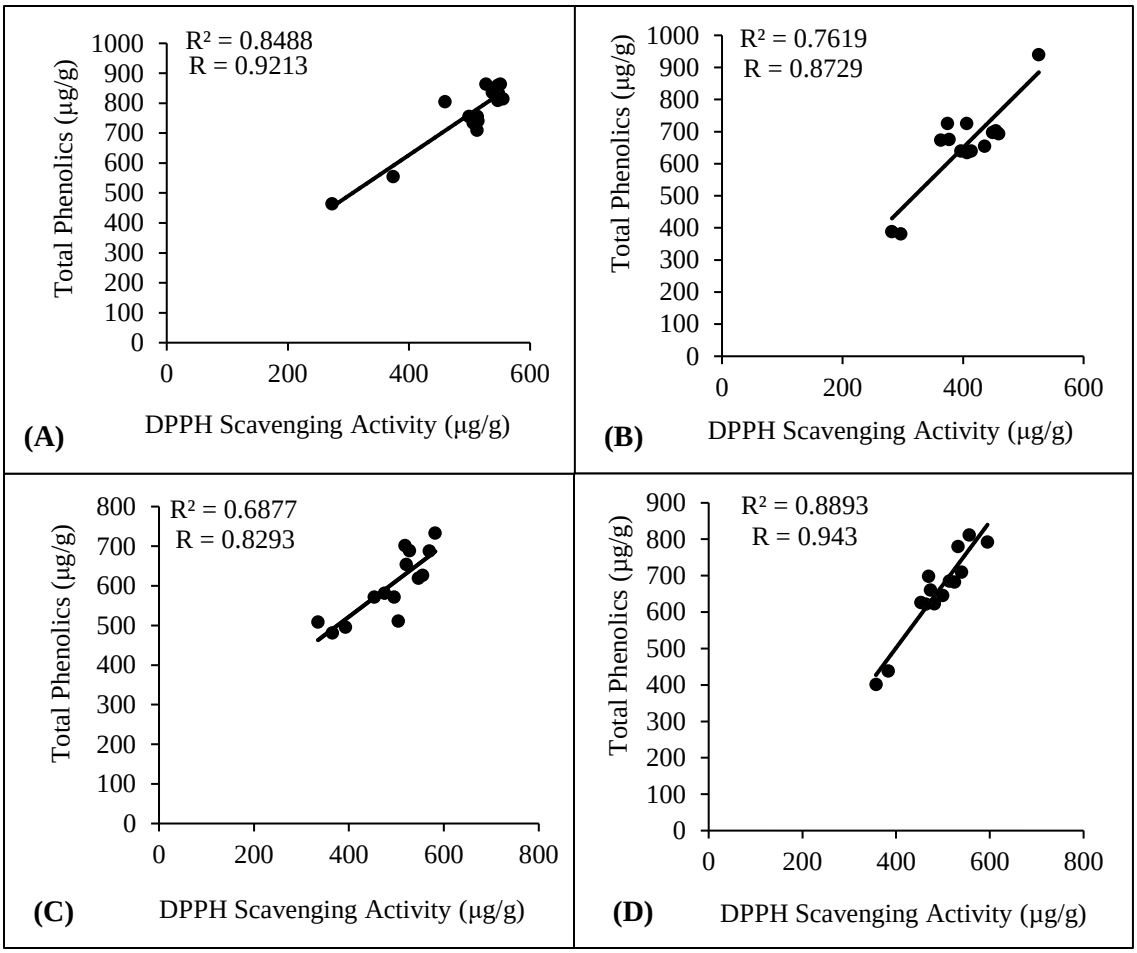


**Figure 10. Linear regression($R^2$) and Pearson correlation coefficients (R) between DPPH scavenging activities and total phenolics in solvent, water, and acid extraction solvent combinations with (A) FA: formic acid, (B) HCl: hydrochloric acid, (C) AA: acetic acid and (D): citric acid.**

*Ascorbic acid, dehydroascorbic acid and total ascorbic acid*

Garnet Stem dandelions, harvested from two different locations, Texas (**Fig. 6a,**) and New Jersey (**Fig. 6b,**) were evaluated for vitamin C content in the whole leaf, leaf blades, and midvein/petiole (**Fig. 6c.-6e**). Garnet Stem samples from Texas contained no detectable amounts of vitamin C (**Fig. 11.**); however New Jersey leaf blade sample had highest total ascorbic acid (301.9±17.7 µg/g) and dehydroascorbic acid (247.8±23.8 µg/g) as compare to whole leaf and midrib (**Table 9**). Dehydroascorbic acid contributed the most to total ascorbic acid content in the whole leaf, leaf blade and midvein/petiole from New Jersey. The finding of no detectable amounts of vitamin C in Garnet Stem samples from Texas could be due to environmental stress, temperature, soil, pre- and post-harvest practices, storage conditions, etc.[18, 36, 109-112] To confirm no detectable amounts of ascorbic acid in Garnet Stem from Texas, extracted ion chromatograms of standard ascorbic acid, Garnet Stem from New Jersey, and Garnet Stem form Texas were completed by UV absorption spectra and electrospray positive ionization on UPLC-HR-QTOFMS (**Fig. 11.**).

Studies have demonstrated that high nitrogen present in soils could decrease vitamin C, light intensity could alter the amount of vitamin C present, conditions of high water loss could decrease vitamin C, and high temperatures inflicted on crops after harvest could degrade vitamin C.[109, 113] Another study reported that with increasing concentrations of copper in the soil, an increase in vitamin C occurred.[36] Due to the vast geographical difference between the harvest areas, location could be a major factor for the non-detectable amounts of vitamin C in the Garnet Stem samples from Texas. We conclude that more experimental and analytical investigations will need to be conducted to examine vitamin C in Garnet Stem dandelions from Texas.

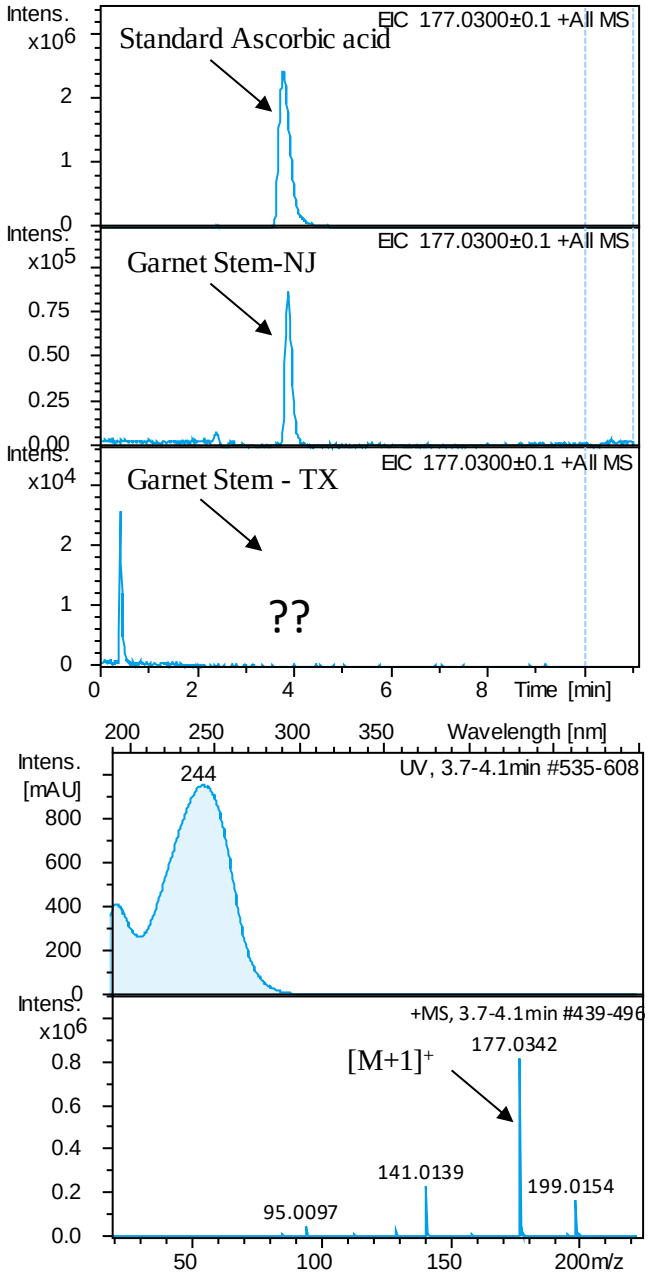


**Figure 11. Extracted ion chromatograms of standard ascorbic acid and Garnet Stem from New Jersey and Texas, UV absorption spectra and electrospray positive ionization of ascorbic acid.**

*Carotenoids and chlorophylls*

Three carotenoids (lutein, β-carotene, and violaxanthin) and chlorophyll a and chlorophyll b were identified in the Garnet Stem samples harvested from Texas and New Jersey (**Table 9**). Garnet Stem leaf blades from Texas and New Jersey, contained significantly different amounts of carotenoids, chlorophyll a and chlorophyll b (except for β-carotene) compared with the whole leaf and midvein/petiole tissue ($p<0.05$) (**Table 9**). Both chlorophyll a and chlorophyll b were doubled in amount in the leaf blade versus the whole leaf. Texas samples contained 270.7±24.4 µg/g chlorophyll a and 285.9±26.8 µg/g chlorophyll b and New Jersey samples contained 67.5±14.0 µg/g chlorophyll a and 65.1±13.2 µg/g chlorophyll b in the whole leaf. In the leaf blade, Texas samples contained 404.7±32.7 µg/g chlorophyll a and 420.9±32.6 µg/g chlorophyll b and New Jersey samples contained 128.0±2.0 µg/g chlorophyll a and 125.2±15.3 µg/g chlorophyll b.

Location and climate of Garnet Stem samples from Texas resulted in significant differences in chlorophyll ($p<0.05$). The UV-A and UV-B from sunlight could increase carotenoid and chlorophyll levels, depending on exposure.[114, 115] Compared with other leafy greens such as kale, the Garnet Stem samples had lower amounts of chlorophyll a.[116] After two consecutive full years of harvest, kale varieties contained an average of 155.5 and 132.2 mg/100g of chlorophyll a and 45.1 and 32.9 mg/100g of chlorophyll b.[116] These values are higher compared to Garnet Stem from Texas except for chlorophyll b 420.9±32.6 µg/g (**Table 3**).[116]

Both chlorophyll a and chlorophyll b has been demonstrated for the inhibition of colon cancer cells and liver detoxification.[117, 118] A previous study showed that dandelion

leaves harvested from Slovenia had higher concentrations of β-carotene and chlorophyll a and b, but lower amounts of lutein and violaxanthin [53] compared to the Garnet Stem from Texas and New Jersey (**Table 3**). The lutein from Garnet Stem could also aid with eye health due to higher amount, which can decrease the risk of age-related macular degeneration.[119]

**Table 9. Phytochemicals of Garnet Stem samples grown in different locations**

| Analysis | Sample* | Phytochemical | Texas | New Jersey |
|---|---|---|---|---|
| Total Vitamin C (μg/mL) | Whole Leaf | AA | ND | 49.6±12.7$^{a}$ |
| | | DHA | ND | 169.9±16.2$^{a}$ |
| | | TA | ND | 219.6±15.6$^{b}$ |
| | Leaf Blade | AA | ND | 54.1±3.7$^{a}$ |
| | | DHA | ND | 247.8±23.8$^{a}$ |
| | | TA | ND | 301.9±17.7$^{a}$ |
| | Midvein/ Petiole | AA | ND | 26.9±2.4$^{a}$ |
| | | DHA | ND | 35.1±5.7$^{b}$ |
| | | TA | ND | 62.1±3.8$^{c}$ |
| Carotenoids and Chlorophylls (μg/g) | Whole leaf | Lutein | 36.7±2.1$^{b}$ | 18.1±6.1$^{b}$ |
| | | β-Carotene | 3.8±0.2$^{a}$ | 13.1±2.7$^{a}$ |
| | | Violaxanthin | 9.0±0.7$^{b}$ | 1.8±1.3$^{ab}$ |
| | | Chlorophyll a | 270.7±18.3$^{b}$ | 67.5±14.0$^{b}$ |
| | | Chlorophyll b | 285.9±17.9$^{b}$ | 65.1±13.2$^{b}$ |
| | Leaf Blade | Lutein | 57.4±4.6$^{a}$ | 46.0±7.3$^{a}$ |
| | | β-Carotene | 2.4±0.2$^{ab}$ | 28.7±4.9$^{a}$ |
| | | Violaxanthin | 15.3±0.6$^{a}$ | 3.5±1.7$^{a}$ |
| | | Chlorophyll a | 404.7±41.1$^{a}$ | 128.0±2.0$^{a}$ |
| | | Chlorophyll b | 420.9±41.0$^{a}$ | 125.2±5.3$^{a}$ |
| | Midvein/ Petiole | Lutein | 3.0±0.1$^{c}$ | 1.5±0.6$^{c}$ |
| | | β-Carotene | 1.4±0.1$^{b}$ | 0.1±0.3$^{c}$ |
| | | Violaxanthin | 0.1±0.08$^{c}$ | 0.0±0.0$^{b}$ |
| | | Chlorophyll a | 28.7±1.1$^{c}$ | 19.0±2.6$^{c}$ |
| | | Chlorophyll b | 37.6±1.7$^{c}$ | 20.1±2.3$^{c}$ |

**ND: not detectable, AA: ascorbic acid, DHA: dehydroascorbic acid, and TA: total ascorbic acid**
**Results are presented as mean ± SE from triplicates samples**
**Different alphabets within the column indicates the significant differences between different plant materials (ANOVA, $p<0.05$)**

*Determination of bile acid binding capacity and dietary fiber*

Six bile acid salts were used to determine the bile acid binding capacity of Garnet Stem whole leaf samples from Texas and New Jersey. These samples were able to bind to all six bile acid salts with the highest capacity for binding CDCA and DCA (**Fig. 12.**). According to a previous study, the bile salts CDCA and DCA were toxic if produced at high concentrations within our bodies.[85] Both CDCA and DCA have low critical micellization concentrations, meaning the micelle surface interface saturation is lower than that of other bile acid salts; lipid saturation of CDCA and DCA is lower thus giving them a higher chance of becoming cytotoxic if oversaturated.[85] The binding capapcities for all six bile acid salts were not significantly different between samples from Texas and New Jersey. Binding of bile acid salts may result from the total dietary fiber present in Garnet Stem samples. Texas had the highest total dietary fiber (44.1%), soluble dietary fiber (10.4%) and insoluble dietary fiber (33.7%) versus Garnet Stem from New Jersey (35.4% total, 8.7% soluble and 26.7% insoluble). Soluble and insoluble dietary fiber have many effects and binding of bile acid salts likely is one of the mechanisms.[88] According to the chemical composition and mechanism of action of soluble fiber, it is responsible for binding bile acid salts.[87, 88] Soluble dietary fiber, also known as viscous dietary fiber, can slow down digestion by slowing diffusion of digestion products.[87] The soluble fiber in dandelion is mostly (12–15%) inulin β(2→1) fructan gel-like substances, which can dissolve and thicken in water. These compounds can reduce absorption of glucose and lower insulin responses by stabilizing and controlling the absorption of sugar and affect the synthesis of lipids.[87-89] Soluble fiber can enhance digestion by binding to bile acid salts

and eliminating them from the body, thereby reducing serum cholesterol.[87-89] Insoluble dietary fiber absorbs water and any substances in the colon, which then pass through the digestive system.[87] Further, dandelion dietary fiber binding capacity and removal of toxic waste from the body is not fully uunderstood.

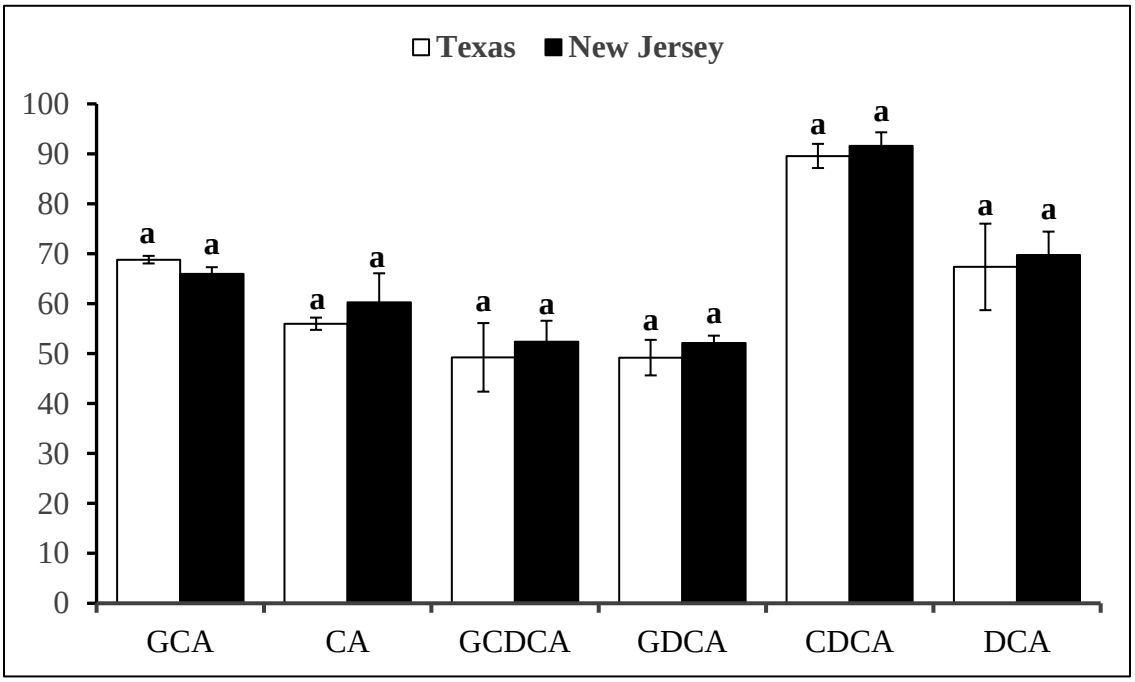


**Figure 12. Bile acids binding capacity of Garnet Stem whole leaf samples from Texas and New Jersey. Different letters indicate a significant difference of binding for each bile acid (ANOVA, $p<0.05$).**

## Chapter Summary

In conclusion, this study focused on the extraction efficiency, quantification, and identification of anthocyanins in the red midvein and petiole tissue of Garnet Stem dandelions, using 28 different solvent combinations. Four anthocyanins, cyanidin-3-glucoside, cyanidin-3-(6-malonyl)-glucoside (A-1), cyanidin-3-(6-malonyl)-glucoside (A-2) and peonidin-3-(malonyl)-glucoside, were extracted at maximum level was found in MeOH: FA (99:1). DPPH scavenging activity was highest with MeOH: water: CA (80: 19: 1) and total phenolics were highest with EtOH: water: HCl (50: 49: 1). The levels of anthocyanins and other phytochemicals differed depending on the harvest location and the part of the plant. Phytochemical contents differed depending on the harvest location and sample part of Garnet Stem. These findings also indicate multiple routes for continuation of this research to explore the use the anthocyanin extracts as c colourlants for food formulations.

# CHAPTER IV

# CONCLUSION

Dandelion is currently being underutilized as a leafy green vegetable for its health promoting compounds. Consumers perceptions of dandelion as a weed is neglecting dandelions potential as another source of antioxidant activity, diuretic aid, hepatoprotective aid, dietary fiber, heart health benefits, and various sources of vitamins and minerals. Dandelion roots and bitter leafy greens leaves has been traditionally used by Native Americans, Europeans in Mediterranean countries and Asian countries to help treat disease and illness associated with the liver, kidneys, heart, stomach and etc by consumption. Various ways of consuming dandelion are by boiling the roots and leaves into herbal teas and tonics and eating the dandelion leaves raw for many health benefits.

Catalonga and Garnet Stem dandelion varieties from Texas and New Jersey were separated in whole leaf, leaf blade and petiole and evaluated for phytochemicals, antioxidant activity by different thermal techniques, bile acid binding capacity and dietary fiber. Catalonga from Texas contained no detectable amounts of vitamin C during the early harvest while Catalonga from New Jersey did during the early and late harvest, Garnet Stem's red midvein/petiole tissue contains four anthocyanins which express DPPH scavenging activity and total phenolics that were first to be reported, the anthocyanin extracts that best extracted with MeOH: FA (99: 1) are a bright red/pink color which have the potential to be used as a natural red colorant in food products, boiling or microwaving dandelion greens, especially Garnet Stem variety, for 15 min and 4 min is sufficient to obtain highest levels of antioxidants in the form of an herbal

tea, both Catalonga and Garnet Stem varieties were able to bind to six different bile acid salts with highest level of binding to CDCA and DCA, which can be toxic to humans if accumulated at high concentrations within the body, and Catalonga from Texas and Garnet Stem from New Jersey had higher total dietary fiber.

These results amongst the rest, confirm that based on location and harvesting time, there are differences in the levels of health promoting compounds of dandelion Catalonga and Garnet Stem varieties. Also, between whole leaf, leaf blade and midvein/petiole, the fully matured leaf blade overall had the highest amounts of phytochemicals, DPPH radical scavenging activity, and total phenolics. Further studies can use these results as a foundation to research dandelion leaf blades for liver, kidney, cardiovascular, gastric, intestinal, and other related diseases.

Dandelions dietary fiber role in reducing cholesterol and aiding cardiovascular health could also be further examined. Bioavailability of these quantified phytochemicals, especially Garnet Stem's red midrib/petiole anthocyanin, could be further analyzed to prevent oxidative stress related illnesses. Garnet Stem's red midrib/petiole extract has the potential be used in food products as a natural red colorant and should be tested for its stability. Lastly, dandelion hot herbal leafy green teas could be compared to cold-pressed dandelion green juice to determine if heat or non-heated dandelion greens have different levels of antioxidants and bitter sesquiterpene lactones and terpenoids.

The potential for dandelion leafy green research can continue to be explored through many routes. Dandelions have the potential to become more available in

produce markets of grocery stores if its research is continued to inform consumers of its health benefitting compounds. Dandelion leafy greens have the potential to prevent the onset of disease and illness, help aid growers to increase yield of their dandelion crop, and bring awareness and educated consumers of the multiple ways dandelion can be consumed as a food product such as a fresh salad, savory side dish and/or herbal detoxifying tea.

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