Abstract
Phenolic compounds have been extensively studied due to their antioxidant, anti-carcinogenic, anti-inflammatory and antimicrobial properties. Each plant or plant product can be characterized by its unique phenolic profile. However, these extracts are a complex mixture of phenolic compounds, sugars, fatty acids, and proteins. The large number of compounds and the variety of structures and properties require a high throughput, robust, and customizable system must be used. Mass spectrometry, which can be coupled to chromatography columns, and used with a variety of ionizers, mass analyzers, and detectors, is an ideal technique, with HPLC-ESI-QTOF-MS, in particular, becoming quite popular due to its customizable system, readily available standards, and growing list of published protocols.

Keywords: Diode Array Detection; Electrospray Ionization; High Performance Liquid Chromatography; Mass Spectrometry; Plant Biochemistry; Phenolic Compounds; Phytochemicals

Introduction
Phenolic compounds contain an -OH (hydroxyl) group conjugated to an aromatic ring and are often associated with the pharmacological activity, such as antioxidant properties, in plants [1-6]. This makes their quantification and identification a critical part of the search for new active compounds for the treatment and prevention of diseases [7,8]. In crop science, phenolic profiles may be used to characterize plant sources and environmental conditions and to study the impact of chemicals on plants [4]. In food science, phenolic compounds can be used to replace synthetic additives that extend shelf-life and phenolic profiling may be used to identify specific foods that confer health benefits such as disease prevention [4]. Consequently, phenolic profiles are of great interest to members of the research community, pharmaceutical industries, food quality and inspection agencies and physicians searching for cures to diseases.

The study of phenolics requires a high throughput, high resolution method for generating phenolic profiles from complex mixtures, such as mass spectrometry (MS) [9]. In MS, the analysis of the fragmentation patterns of ionized gases has been used to quickly characterize the compounds found in plant extracts [4]. By coupling chromatography columns to mass spectrometers, researchers are able to use both retention time and ion Mass to Charge (m/z) ratios to characterize the components of a complex mixture, such as plant extracts [4]. These columns may also be connected to UV-vis detectors for additional information [4]. This enables researchers to analyze one sample by many detectors in tandem generating high quality data. The high cost, complexity of the spectra, and necessity of optimization have not stopped this technique from quickly gaining popularity with researchers in a variety of fields.

This paper will briefly summarize recent discoveries, methodologies, and applications of MS in studies of phenolic compounds in 2016, for a brief summary of 2015 research please refer to other literature such as Parasram, K. 2015 [10].

Mass Spectrometry Techniques
Compared to GC-MS, LC and HPLC-MS does not require prior derivatization [11]. Liquid Chromatography/LC or High Performance Liquid Chromatography (HPLC) with Diode Array Detectors (DAD) coupled to electrospray ionization (ESI) or Matrix-Assisted Laser Desorption Ionization (MALDI) and Time of Flight (TOF) is a common approach to phenolic profiling due to the readily available standards and established protocols for chromatography [4,6,12-14]. Orbitrap, quadrupole, and ion trap analyzers also have been used instead of TOF [4,14-16]. Capillary Electrophoresis (CE-MS) has been used for separation of highly polar, charged molecules that do not interact sufficiently with standard reverse phase LC columns [17]. These experiments can be performed with negative ions or positive ions to identify different types of phenolics [4,18]. This method may also be coupled with UV-vis detection resulting in three distinct results, characteristic of each compound in the mixture: Rt (Retention time on the chromatography column), m/z (mass to charge ratio from the mass spectrometer), and λ max (wavelength(s) of absorption from UV-vis) [4,14]. Identification of the compounds based on these three results is more reliable than use of one method alone. However, by coupling chromatography columns to mass spectrometers there is the potential for matrix effects to cause ion suppression or enhancement which causes false positives or false negatives which may make experiments difficult to replicate [12].

HPLC-MS
HPLC-ESI-MS experiments use a variety of analyzers: TOF, ion trap, Orbitrap, and quadrupole/TOF are some examples (figure 1) [4,6,14-16]. In order to select the ideal mass analyzer the molecular weight of the compound of interest, the desired resolution and selectivity, and expected fragmentation should be considered. Quadrupole analyzers provide superior high resolution which may be useful when quantifying and identifying new compounds and can be combined with TOF to increase the resolution of conventional TOF analyzers [4,6,14-16]. Ion trap, including Orbitrap, allow exceptional ion selectivity, for in depth study of specific ions (compounds), whereas TOF is a less expensive and sufficiently high resolution and selectivity option for analysis ideal for profiling a large range of compounds with diverse structures, such as the phenolic profile of a plant [4,6,14-16]. One study comparing
Recent Studies

LC-ESI-MS (table 1) was used to study tea, sesame oil, red lettuce, pitomba, argan, salsify, Turkish thyme, Alcaea pallida, Alcaea apterocarpa, red raspberry, and cucurbitaceae seeds [5,13,22,24-31]. HPLC and UHPLC are used to improve the separation and decrease the time to obtain results [23]. HPLC-ESI-MS (table 1) has been used to study blackcurrant cultivars, caper berries, doudberries, cherries, cranberries, kale, red cabbage, basil, sword bean, Easter pear, mistletoe, fermented strawberry drink, tomatoes, lettuce, ginger, Bidens pilosa, apple cider and blackberry wine [1-3,11,14,16,23,32-37]. Studies of sesame oil, Alcaea pallida, Alcaea apterocarpa, salsify, argan, pitomba, black currants, Bidens pilosa, Turkish thyme, lettuce, tomatoes, fermented strawberry drink, mistletoe, Easter pear, cherries, cranberries and sword beans were performed with the goal of identifying the phenolic content of plant or fruit extracts [1,3,11,13,23,25,27-32,36,37]. Where studies of cloudberry and caper berry used fermentation, the study of tea, ginger, kale and red cabbage focused on the effect of cooking method and studies of basil, apple cider, and blackberry wine were used to optimize mass spectrometry procedures [2,5,14,16,31,33-35]. These studies show that LC-MS, HPLC-MS and UHPLC-MS can all be used to achieve similar experimental goals therefore the selection of an appropriate technique is dependent upon the complexity of the mixture and the desired level of resolution.

The study of the effect of temperature and day length on the phenolic content of black currant berries used HPLC-ESI-Ion Trap-MS and found that lower temperatures and high light generate high quality berries [23]. CO₂ enrichment of crops is used to increase yields of greenhouse crops; the effect of CO₂ enrichment on phenolic content of red lettuce leaves was studied using HPLC-ESI-Ion trap-MS [26]. Researchers found that high CO₂ concentration increased levels of sugar, flavonoid glycoside and, non-uniformly, caffeic acid derivatives, demonstrating that CO₂ enrichment may result in phenolic-rich red lettuce crops [26]. LC-ESI-MS was used to study the phenolic composition of argan leaves and used complementary data from UV-vis readings to identify 13 phenolic compounds and quantify seven [30]. A combination of LC and GC-
MS was used to identify the components of the phenolic profile and aroma of the pitomba fruit, respectively, and successful identified 13 phenolic compounds and 27 volatile compounds, identifying quinic acid as the main component of the phenolic profile [25]. Desorption ESI (DESI) MS was used to generate at 2-D map of the coffee bean endosperm revealing that quinic acid and feruloylquinic acid were found on the external endosperm, feruloylquinic acid on the internal endosperm, and caffeoylquinopolyquinic acid in the injured endosperm region [38]. Ginger preparation was studied using UPLC-QTOF-MS and found that dried ginger had the highest phenolic content, followed by stir-fried ginger, fresh ginger, and carbonized ginger [33]. Turkish tea was studied to determine the effect of infusion time and temperature on phenolic content and antioxidant activity and was found to increase with increasing time, optimal at 10 minutes, and temperature, optimal at 100°C, with LC-MS/MS detecting gallic acids, flavan-3-ols, quinic esters, flavonoids, theaflavins and purine alkaloids [5]. The study of okra using MALDI-TOF-MS and HPLC-MS/MS revealed that amylase and glucosidase inhibition are caused by proanthocyanidins in unripe okra seeds, and MALDI-TOF spectra was used to identify B-type gallocatechin oligomers up to 15 in length based on their singly charged molecular ion [21].

**Conclusion**

The use of mass spectrometry in the study of phenolic compounds in plants has generated a large number of techniques, the most popular being HPLC-ESI-MS. The studies conducted in recent years demonstrate a shift from the use of a single MS technique to the combination of columns, 2-D LC MS, or techniques, HPLC-MS and MALDI MS, to accomplish a single goal. The established protocols and growing mass spectra databases enable researchers to address both bioactivities and characterization of phenolic content more easily than ever before. The applications of mass spectrometry research of phenolic compounds are varied ranging from pharmaceutical industries, to agriculture and food safety. Future optimization of MS techniques and development of 2-D MS and 2-D imaging, such as DESI-MS, techniques serve to advance the field of mass spectrometry for phenolic compound research.

**References**


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Received Date: April 14, 2016, Accepted Date: June 08, 2016, Published Date: June 17, 2016.

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