# Analysis of Phenolic Compounds Extracted from Peanut Seed Testa

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*Peanuts (Arachis hypogaea) contain numerous phenolic compounds with antimicrobial and antioxidant properties. These secondary metabolites may be isolated as co-products from peanut skins or testae during peanut processing and have potential use in functional food or feed formulations. Peanut skins were extracted in ethanol and analyzed by mass spectrometry and ultraviolet spectroscopy to identify major phenolic compounds. Extracts were analyzed by LC-MS (Accela-MSQ, ThermoFisher Scientific,Waltham, Massachusetts, USA). Separations were performed using a PFP column in reverse phase. The MS detector was scanned from 50 – 500 m/z in negative mode (ESI). Additional analyses of extracts were performed by GC-MS (Polaris MSQ, ThermoFisher Scientific,Waltham, Massachusetts, USA), ultraviolet spectroscopy, and fluorescence spectroscopy. Data were used to obtain a profile of the phenolic compounds that included catechin, epicatechin, and several anthocyanidins. These results are expected to provide a rapid analysis and promote the use of phenolics obtained from peanut skins.*

**Keywords:** anthocyanidins, *Arachis hypogaea*, catechin, peanut skins, spectrometry

## Introduction

Peanuts, *Arachis hypogaea*, are cultivated as a source of edible seed oil and protein. In the United States 1.638 million acres were planted in 2012 with a value over 1 billion dollars according to the USDA, Economic Research Service [1]. Peanut seeds contain 47 wt% oil with a nominal fatty acid composition of 30% linoleic acid, 45% oleic acid, and 20% stearic acid. Peanut seed oil is 75% unsaturated with omega 6 and omega 9 fatty acids. During processing the peanut seed testa or skin that surrounds the seed is typically removed after the shelling process by blanching. The skin is a low value by-product with few applications beyond animal feed although efforts have been made on new product development [2]. Peanut skins contain bioactive phenolic compounds such as catechin and epicatechin [3]. Recent awareness of the health benefits of antioxidant phenolic compounds have generated interest in peanut skins as an economical source. The availability of peanut skins and the associated antioxidant properties of peanut skin phenolic compounds suggests numerous applications in functional food and feed formulations [4-6].

A simple technique for the analysis of phenolic compounds in peanut skins will facilitate the utilization of this agricultural material for antioxidant compounds. Phenolic compounds exhibit strong absorbance in the ultraviolet (UV) region. The absorbance is produced by the aromatic ring structure and often monitored at 280 nm for many phenolic structures. The absorbance is due to electronic transitions and scanning a phenolic compound will produce the corresponding UV absorption spectra. UV spectroscopy offers a relatively simple approach to detect the presence of phenolic compounds, however, the spectra do not exhibit the variation or detail available from vibrational spectroscopy that can help determine the chemical structure of a particular phenolic compound.

The current investigation was undertaken to evaluate spectroscopic and spectrometric techniques for the rapid measurement of peanut skin phenolics. Mass spectrometry can provide detailed structural information to identify closely related phenolic compounds. However, sample preparation and long analysis times are often needed when using liquid or gas chromatography with mass spectrometry. In contrast, spectroscopic methods are rapid, nondestructive, and easily implemented for routine analysis with the development of chemometric models [7,8].

## Methods

*Materials* Blanched peanut skins were obtained from a commercial source (Universal Blanchers, Sylvester, Georgia, USA). Anhydrous ethanol, 200 proof, was purchased from Aaper Alcohol and Chemical Co. (Shelbyville, KY USA). Deionized water, 18 MΩ-cm, was prepared with a laboratory water purification system (Aquasolutions, Jasper, Georgia, USA). Solutions of aqueous alcohol were used for solvent extraction at compositions of 100% ethanol, 80% ethanol, and 100% water,. Extractions were performed with 5 gram samples of peanut skins stirred in 500 mL of solvent at room temperature for 24 hours in the dark.

*LC-MS Analysis* Samples were dissolved in ethanol and analyzed by LC-MS (Accela-MSQ, ThermoFisher Scientific,Waltham, Massachusetts, USA). Separations were performed using a PFP column. The mobile phase was aqueous acetonitrile with 0.1% formic acid. A linear gradient from 10% to 20% acentonitrile was used with a flow rate of 0.4 mL/min and 5 microliter injection volumes. The MS detector was scanned from 50 – 500 m/z in negative mode (ESI).

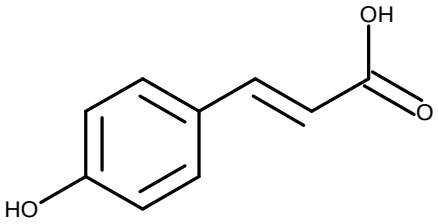
*GC-MS Analysis* Silyl derivatives were prepared with BSTFA (Supelco, Bellefonte, PA USA) by dissolving 5 mg samples in acetone and mixing with the reagant at room temperature. Analysis was performed by GC-MS with an ion trap detector (Polaris MSQ, ThermoFisher Scientific,Waltham, Massachusetts, USA). Injection volumes of 1 microliter were made, splitless, on a 60 m DB-5ms column (J&W Scientific, Agilent Technologies, Santa Clara, CA USA) with the oven temperature program starting at 100°C increasing to 200°C at 10°C/min. The inlet and transfer line were 250°C with helium carrier gas flow 1.5 ml/min. The detector was operated in selective ion mode (SIM).

*Spectroscopic analysis* Standards were prepared from 98% pure catechin, epicatechin, ferulic acid, *p*-coumaric acid, 3,5-dimethoxy-4-methoxy cinnamic acid and 3,4-dihydroxy cinnamic acid (Sigma-Aldrich, St. Louis, MO, USA). Ultraviolet spectra were collected on a Perkin Elmer model Lambda 2S UV/VIS spectrometer (Waltham, MA, USA). Standards were diluted to 1, 5, and 10 µg/mL and scanned in 3.5 mL quartz cuvettes. Spectra were collected from 200-400 nm at 240 nm/min with a 2 nm slit width. Emission spectra were collected with a Perkin Elmer model LS-55 luminescence spectrometer (Waltham, MA, USA). Samples were excited at 280 nm with emission spectra collected from 300-400 nm. Spectra were processed with the chemometric software Unscrambler X (Camo Software, Oslo, Norway). Derivative spectra were calculated by the Savitsky-Golay method. Principal component analysis (PCA) was performed on smoothed and first derivative spectra to determine potential grouping for subsequent classification [9].

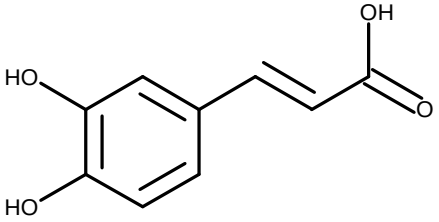
**Results and Discussion**

Chromatographic techniques, particularly liquid chromatography, are useful for the analysis of natural products such as plant phenolics. The analytes are generally obtained by extraction and derivatization is not required. Because phenolics absorb strongly in the UV region a diode array detector is useful and often combined with mass spectrometry to determine the amount and structure of compounds in a sample. However, analysis times of 20 minutes are typically needed to resolve a mixture of phenolics. Gradient elution separated catechin from epicatechin within 10 minutes and 5 anthocyanidins compounds after an additional 10 minutes of analysis time. For comparison gas chromatography was performed after forming the silyl derivatives. The gc-ms method offered no significant advantage to lc-ms since the analysis times were similar and derivatives had to be made. In contrast, spectroscopic methods are more rapid and no sample preparation is necessary. Catechin and epicatechin absorb strongly at 280 nm and

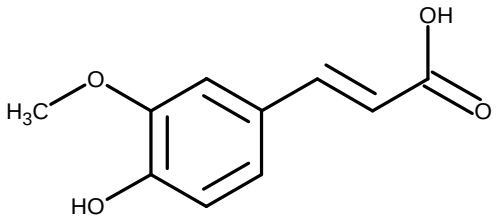
exhibit emission at 315 nm when excited. Ultraviolet spectra were collected from mixtures of catechin and small phenolic acids, e. g., ferulic acid and coumaric acid, to determine how these compounds could be resolved with chemometric techniques. The chemical structures are shown in Figure 1.



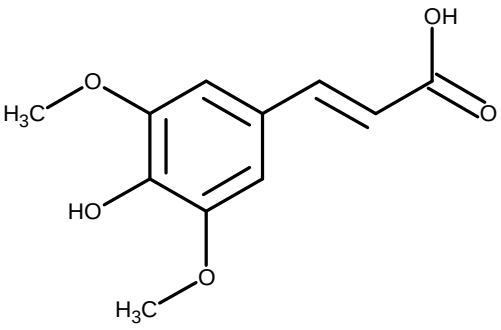

***p*-coumaric acid**




**3,4-dihydroxycinnamic acid**




**Ferulic acid**




**3,5-dimethoxy-4-hydroxycinnamic acid**

Figure 1. Related small phenolic acid structures.

The UV spectra of ferulic acid and *p*-coumaric acid, for example, show clear differences over the scan range of 200-400 nm (Figure 2).

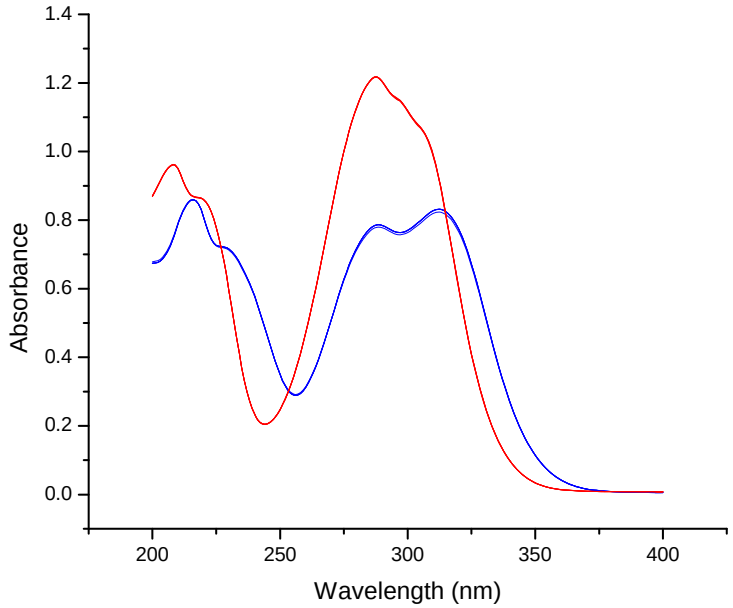



Figure 2. UV spectra of ferulic acid (blue) and *p*-coumaric acid (red).

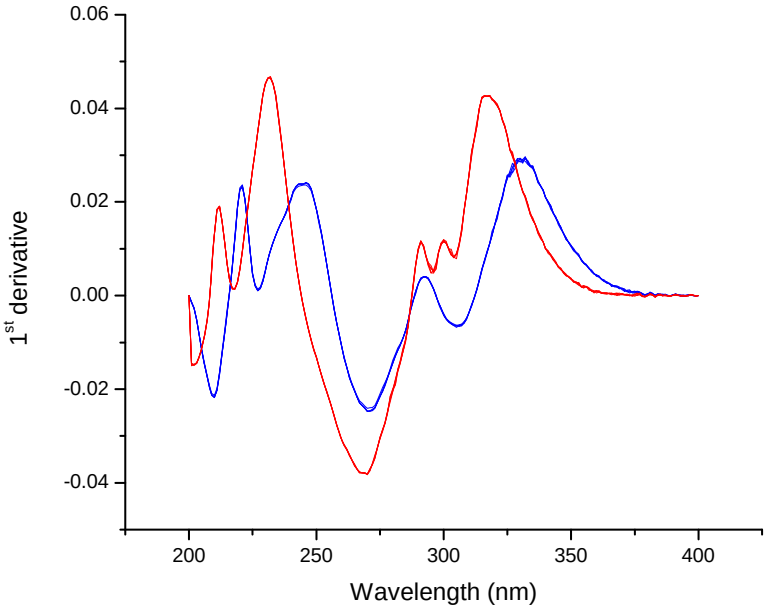



Figure 3. First derivative UV spectra of ferulic acid (blue) and *p*-coumaric acid (red).

Ferulic acid exhibits a maximum absorbance at 215 nm with additional absorbances at 287 nm and 312 nm. In contrast, *p*-coumaric acid displays a maximum absorbance at 286 nm with additional absorbances at 209 nm and 220 nm. Applying the first derivative transform to these spectra produced the results shown in Figure 3. Maxima occur in the derivative spectra at 231 nm and 315 nm for *p*-coumaric acid and 220 nm, 242 nm, and 328 nm for ferulic acid. The UV spectra of catechin and the first derivative transform are shown in Figures 4 and 5, respectively. The spectral region between 230 nm - 310 nm was used for analysis.

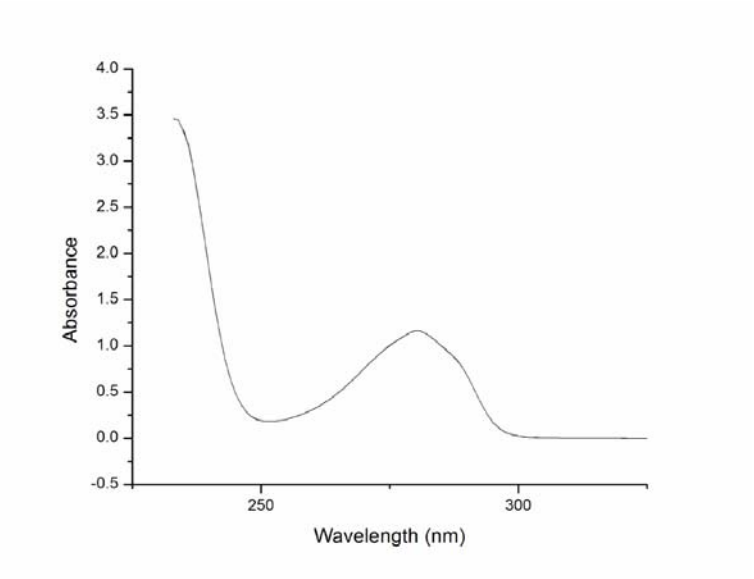



Fig. 4. Catechin UV spectrum.

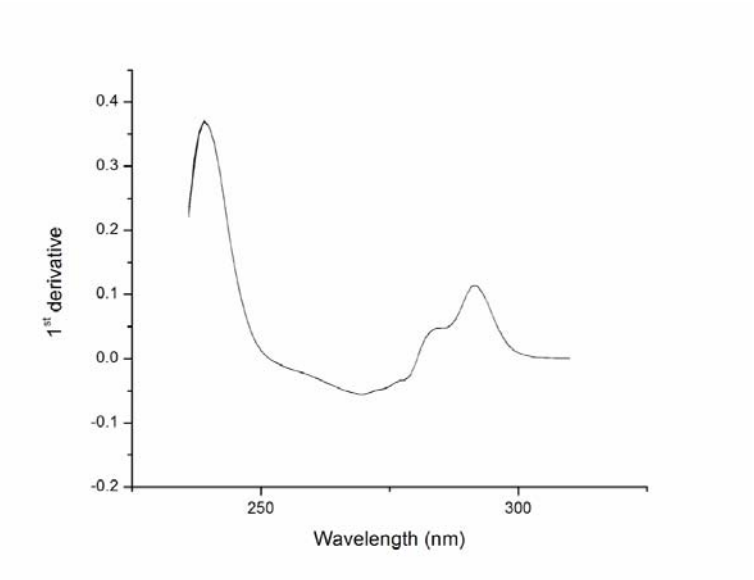



Fig. 5. Catechin 1st derivative UV spectrum.

Spectra were evaluated by principal component analysis (PCA) implemented using the NIPALS algorithm with cross validation and mean centered data. The preliminary PCA results demonstrated the ability to distinguish between catechin, *p*-coumaric acid, and ferulic acid. PCA results for the derivative UV spectra accounted for 99% of the variation with 2 principal components and could be used to classify these phenolic compounds from a mixture of compounds. A more sensitive method exploits the emission spectrum of catechin when excited at 280 nm. The emission spectrum for catechin is shown in Fig 6 with the ethanol peanut skin extract in Fig 7. This technique is very specific for compounds that exhibit fluorescence when there is no significant quenching.

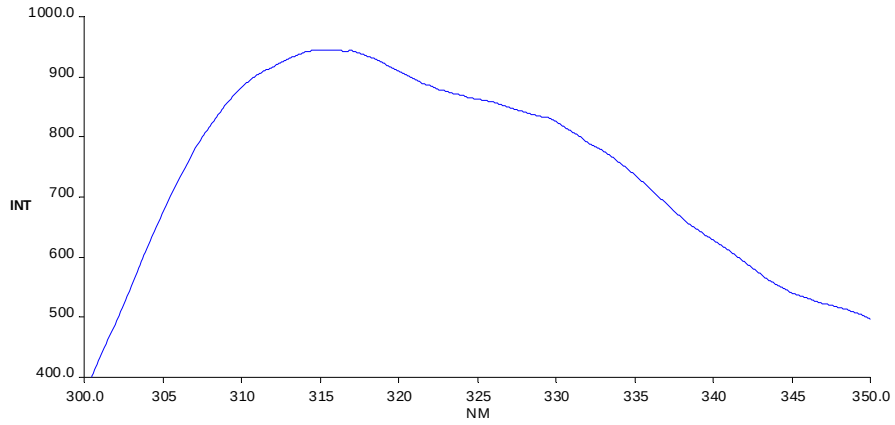



Fig. 6. Catechin emission spectrum.

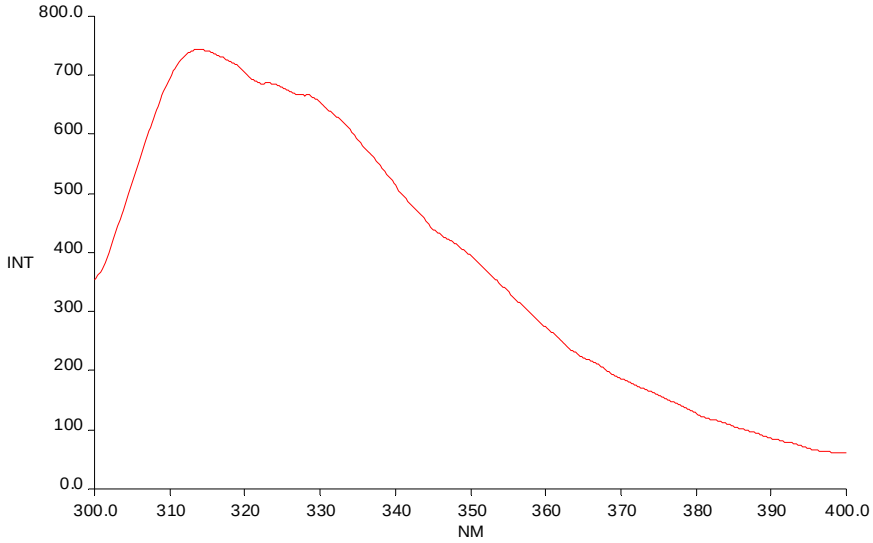



Fig. 7. Peanut skins ethanol extract emission spectrum.

The corresponding derivative spectra are displayed in Figures 8 and 9. The maximum emission wavelength at 315 is observed as a shoulder on the large peak centered near 305 nm. The region above 320 nm is somewhat noisy. However, the use of derivative spectra is not needed for the analysis of catechin in peanut skin extracts.

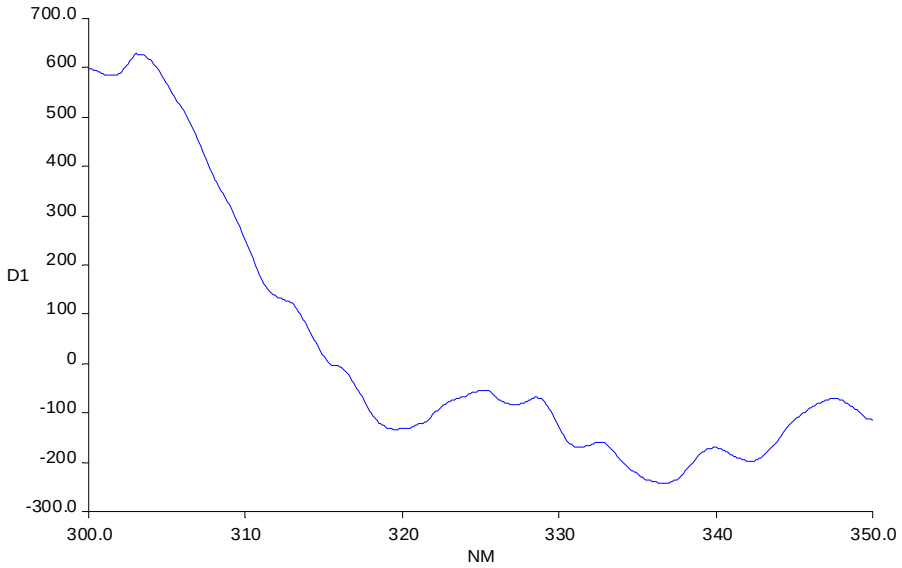



Fig. 8. Catechin 1st derivative emission spectrum.

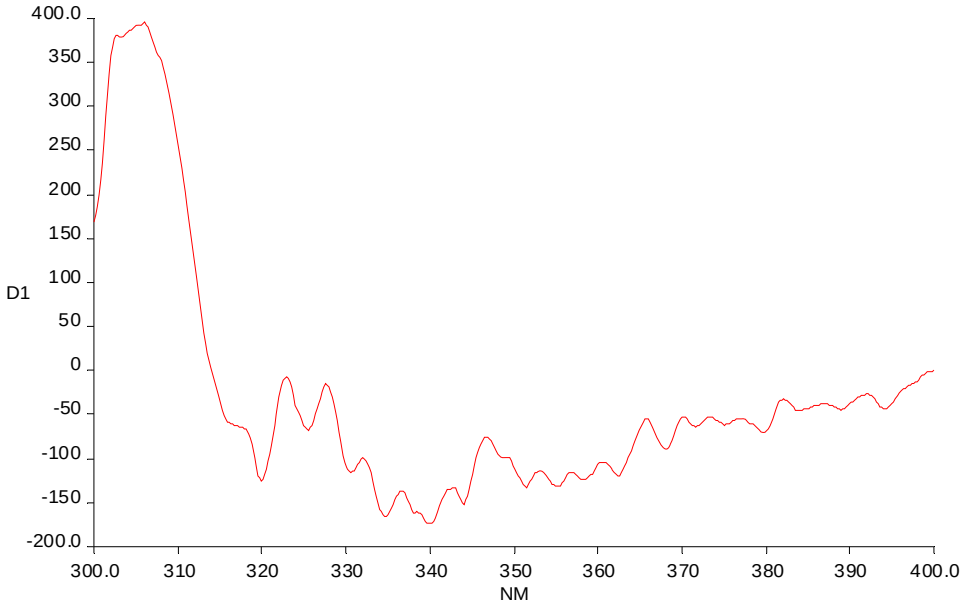



Fig. 9. Peanut skin ethanol extract 1st derivative emission spectrum.

## Conclusions

The application of UV spectroscopy with principal component analysis provided an effective method to classify phenolic acids based on spectral characteristics. Fluorescence spectroscopy was more sensitive than UV for peanut skin phenolic compounds such as catechin. Mass spectrometry was useful for identification of trace compounds especially those compounds generated during the blanching process. However, for routine analysis the spectroscopic methods provide a rapid non-destructive approach to determine phenolic compounds in peanut skin extracts. These results are expected to the use of peanut skin phenolics in functional foods and animal feeds.

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