Identification and characterization of anthocyanins present in banana bract of Indian origin

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1: Introduction

Anthocyanins are a class of natural dyes which are responsible for all attractive hues starting from pink, red, blue to purple. Various sources of anthocyanins have been explored and are used commercially in food products such as soft-drinks, jellies, jams etc. in limited manner. In order to increase the commercial viability of natural dyes, it is necessary to produce these dyes in higher quantity in marketable form. Hence, it is the need of the hour to investigate newer sources of anthocyanins and their detailed analysis is required.

Figure 1. Banana bract

Banana bracts have a strong hue of purple color, which indicates presence of anthocyanins. Moreover, banana bract (shown in Figure 1) are traditionally consumed as food by many without any apparent toxic effects[1]; also, India is world's leading producer of bananas and on this basis, banana bract holds a promising position for extraction of anthocyanins. This plant has been exploited for anthocyanin extraction to a limited extent and very little scientific literature is available regarding the same. Hence, there exists a scope for study of this plant as anthocyanin source. In this poster, anthocyanins from banana bract of Indian origin were identified and characterized using Ultra High Performance Liquid Chromatography (UHPLC) Nexera system coupled with LCMS-8030 triple quadrupole system.

2: Method of Analysis

2-1: Extraction and purification of anthocyanins from banana bract

Banana bracts were collected from Sirsi town in the state of Karnataka. Approximately 50g of banana bracts was ground finely using mixer. The ground bracts were mixed with 0.15%HCl in methanol (v/v) and then filtered using muslin cloth for the extraction of anthocyanins. The same extraction step was repeated until colourless solution was obtained. Filtrates were then combined, evaporated to dryness and finally reconstituted in 3mL of distilled water. This extract was further subjected to Solid Phase Extraction (SPE) for the purification of anthocyanins. The flowchart shown below was followed for the purification of anthocyanins[1].

Condition the C18 SPE cartridge (100mg, 3mL) by passing 6mL of methanol and 0.01% HCl in water consecutively.

↓

Pass aqueous extract through the cartridge.

↓

Wash cartridge with 6mL of 0.01% aqueous HCl to remove sugars, acids and other water-soluble compounds.

↓

Elute with 6mL of methanol containing 0.01% HCl (v/v).

↓

Evaporate the eluate to dryness under the regulated stream of nitrogen gas.

↓

Reconstitute the pigments in 1.5mL of distilled water containing 0.01% HCl.

2-2: LC/MS/MS Analytical Conditions

Figure 2. Nexera with LCMS-8030 triple quadrupole system by Shimadzu

The purified anthocyanin extract was analyzed by LC/MS/MS triple quadrupole system (shown in Figure 2). The details of analytical conditions are given in Table 1.

Table 1. LC/MS/MS analytical conditions

• Column	:	Phenomenex Luna C18(2) HST (50mm L x 3mm I.D. x 2.5µm)
• Mobile phase	:	A: 0.1% trifluoro acetic acid in water B: Methanol
• Flow rate	:	0.5mL/min
• Oven temperature	:	40ºC
• Injection volume	:	50µL
• Detector	:	UV detector at 520nm
• Gradient program (%B)	:	0 - 8min -> 10 - 30%; 8 - 10min -> 30 - 100%; 10 - 12min -> 100%; 12 - 13min -> 100 -10%; 13 - 15min ->10%
• MS interface	:	Electro Spray Ionization (ESI)
• Nitrogen gas flow	:	Nebulizing gas 3L/min; Drying gas 15L/min
• MS temperature	:	Desolvation line 300°C; Heat block 400°C

3: Identification and Characterization of Anthocyanins

The anthocyanins, anthocyanidins with sugar group(s), are mostly 3-glucosides of the anthocyanidins (shown in Figure 3). Identification and characterization of anthocyanins was based on available literature (shown in Table 2). Electrospray mass spectrometry of anthocyanins mainly produces intact molecular ions (M^+). MS^1 analysis of banana bract extract showed molecular ions with m/z ratios of 449, 463, 465, 479, 493, 595, 609, 611, 625 and 639 (shown in Figure 4). These ions may correspond to monohexoside and rutinoside derivatives of cyanidin, peonidin, delphinidin, petunidin and malvidin.

Table 2. Molecular weights of commonly found anthocyanins in banana bract[2]

Anthocyanidins (Acd)	Cyanidin	Peonidin	Delphinidin	Petunidin	Malvidin
Hexose (Hex)	180.2	180.2	180.2	180.2	180.2
Hex-H_2O	162.2	162.2	162.2	162.2	162.2
Acd + 1Hex	449.2	463.2	465.2	479.2	493.2
Rutinose	326.2	326.2	326.2	326.2	326.2
Rutinose-H_2O	308.2	308.2	308.2	308.2	308.2
Acd + Rutinose	595.2	609.2	611.2	625.2	639.2

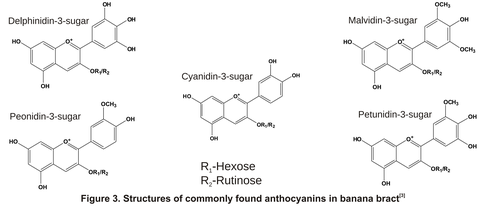


Figure 3. Structures of commonly found anthocyanins in banana bract[2]

MS^2 analysis for above mentioned precursor ions was performed and it resulted in cleavage of glycosidic bonds between flavylium ring and the sugars[2]. The results of MS^2 analysis are shown in Table 3 and product ion spectra are shown in Figure 5.

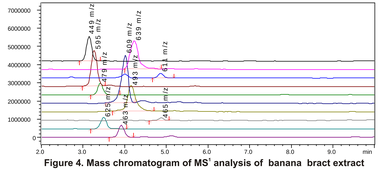


Figure 4. Mass chromatogram of MS^1 analysis of banana bract extract

Table 3. Results of MS^2 analysis of banana bract extract

Precursor Ion m/z	Retention Time (min)	Product Ions m/z	Identified Anthocyanin
449	3.16	287.05	Cyanidin + 1 Hexose
595	3.26	287.00	Cyanidin + Rutinose
479	3.43	316.95	Petunidin + 1 Hexose
625	3.50	317.00	Petunidin + Rutinose
463	3.93	301.00	Peonidin + 1 Hexose
609	4.01	301.00	Peonidin + Rutinose
493	4.15	331.10	Malvidin + 1 Hexose
639	4.24	331.10	Malvidin + Rutinose
611	4.87	303.00	Delphinidin + Rutinose
465	4.88	303.00	Delphinidin + 1 Hexose

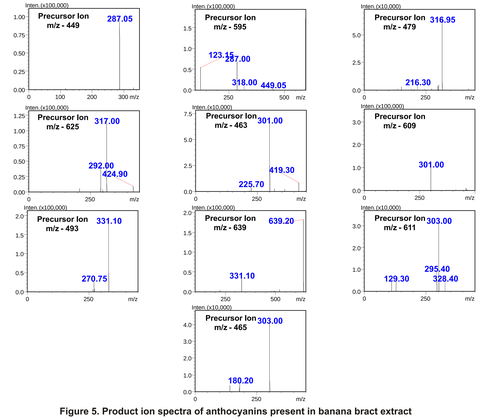
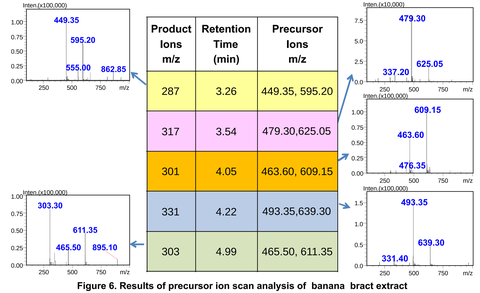


Figure 5. Product ion spectra of anthocyanins present in banana bract extract

Precursor ion scanning is used to confirm the identity of compounds, that result in a common daughter or product ion. The first quadrupole is set to scan the entire mass range that includes all the precursor ions whose fragmentation would result in the selected product ion. The results of precursor ion scan of banana bract extract are shown in Figure 6.



Product Ions m/z	Retention Time (min)	Precursor Ions m/z
287	3.26	449.35, 595.20
317	3.54	479.30,625.05
301	4.05	463.60, 609.15
331	4.22	493.35,639.30
303	4.99	465.50, 611.35

Figure 6. Results of precursor ion scan analysis of banana bract extract

Neutral loss scan allows selective recognition of all ions which, by fragmentation, give the loss of particular neutral fragment. In this case, the neutral losses of two sugar moieties with molecular weight of 162.2Da and 308.2Da were observed. This confirmed the presence of monohexoside and rutinoside derivatives of cyanidin, peonidin, delphinidin, petunidin and malvidin. The results of neutral loss scan are shown in Table 4.

Table 4. Results of neutral loss scan analysis of banana bract extract

Neutral Loss (Da)	Retention Time (min)	Precursor Ions m/z
162.2	3.23	449.20
	3.51	479.25
	3.96	463.25
	4.22	493.25
	4.99	465.35
308.2	3.24	595.25
	3.56	625.40
	4.05	609.20
	4.28	639.35
	4.97	611.30

4: Conclusion

- Fast scanning speed and smaller dwell time of Shimadzu's LCMS-8030 triple quadrupole system enabled analysis in absence of chromatographic separation.
- In addition to product ion scan, precursor ion scan and neutral loss scan prove to be powerful techniques for screening and characterization of anthocyanins.
- The presence of monohexoside and rutinoside derivatives of cyanidin, peonidin, delphinidin, petunidin and malvidin was detected in the banana bract of Indian origin. Banana bract, hence, shows a great potential as an economical source of natural colorant in cosmetic and food products.

5: References

[1] E. Alexandra Pazmiño-Durán et al., Anthocyanins from banana bracts (*Musa X paradisiaca*) as potential food colorants, Food Chemistry, Volume 73, Number 3, (2001), 327-332.

[2] M. Monica Giusti, Luis E. Rodrýguez-Saona et al., Electrospray and Tandem Mass Spectroscopy As Tools for Anthocyanin Characterization, J. Agric. Food Chem. Volume 47, (1999), 4657-4664.

[3] M.C. Lazzé, R. Pizzala et al., Anthocyanins protect against DNA damage induced by tert-butyl-hydroperoxide in rat smooth muscle and hepatoma cells, Mutation Research/Genetic Toxicology and Environmental Mutagenesis, Volume 535, Issue 1, (2003), 103–115.