Research Article

Phenolic profiling of fruit extracts: A discriminative peak analysis through diode array detection in HPLC analysis

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Abstract

An RP-HPLC-PDA method for the simultaneous profiling of phenolic compounds using acidified aqueous phase (pH 2.0) and acetonitrile as an organic modifier was developed. Chromatograms were simultaneously acquired at 210, 254, 280, 320, 370 and 510 nm coupled with PDA detector. Such method of simultaneous and multiple acquisitions help resolving ambiguous peaks and enhancing analytical sensitivity. A standard mixture constituted by the photometric sensitive concentration of each of the 34 compounds resolved in the proposed program within 70 min. The program has been successfully applied to profile phenolic constituents of certain fruit extract *viz., Garcinia pedunculata, Garcinia xanthochymus, Docynia indica, Rhus semialata,* and *Averrhoa carambola*. About 10 phenolic compounds could be successfully quantified for each fruit sample. The proposed RP-HPLC-PDA gradient program is a cost effective method for profiling and quantification of phenolic compositional units that could be applied to various types of sample.

Keywords: RP-HPLC-PDA, Retention factor, Separation factor, Phenolic profile, Wild fruits

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Introduction

Separation, identification and quantification of major phenolic constituents and organic acids present in a fruit is of considerable importance, since these compounds influence organoleptic properties of the product under examination and provide useful information regarding not only its authenticity but also microbial alterations that may have occurred previously [1]. Phenolic compounds are of considerable interest and have received more attention in recent years due to their various bioactive functions. Antioxidant activities of phenolic compounds have been recognized for decades as well as research and developmental activities on the use of natural substances containing phenolic antioxidants will continue to be of great interest to the food industry [2]. Several plant phenolics and fruit extracts have been reported to exhibit anti-inflammatory, anti-carcinogenic, vasodilatory and antimicrobial activities [3-4]. Phenolic compounds constitute a large group of secondary metabolites, though they typically comprise less than 2 % of fresh weight of the plant serving diverse functions such as imparting colour of leaves/fruits, attracting/ repelling insects, antimicrobial/antiviral activity and protecting from harmful radiations and herbivores [2, 5]. The most widely used technique for the isolation and identification of phenolic compounds has been an HPLC system [6] as it enables quantification of native phenolics without any prior transformation. Despites numerous investigations, separation and quantification of different poly-phenolics remain difficult, especially simultaneous determination of poly-phenolics of different groups [7]. In fact, profiling methods are needed that separate and detect all phenolic compounds in a single extract of a food matrix [8]. Simultaneous profiling using HPLC is often limited by various factors such as mobile phase combination, chromatographic support matrices, sample matrix interferences, co-elution problems, differential sensitivity, and relatively wide variation of compositional contents. Additionally, large differences in the level of phenolic compounds in a fruit juice or beverages usually complicated the simultaneous profiling of different classes of phenolic compounds [9].

Present work aimed to establish a cost effective RP-HPLC-PDA method for profiling and quantification of phenolic constituents. An effective extraction protocol for phenolic constituents from fruit sample is designed for use in HPLC analyses. Wild fruits are receiving increased interest to the consumers as well as researchers in evaluating nutritional and medicinal values. Certain wild fruits are consumed and used as an ingredient in formulating herbal remedies for various ailments in many remote areas of the state, Manipur. However, scientific findings about the nutritional and pharmaceutical properties of these wild fruits are very limited. Five such wild fruits *viz., Garcinia*

xanthochymus, Garcinia pedunculata, Docynia indica, Rhus semialata and *Averrhoa carambola* grown in Manipur were taken for study of their phenolic profile and content. To our best knowledge, there is no report on simultaneous profiling of phenolics of these fruits.

Materials and Methods

Chemicals and reagents

Reversed phase test mix (cat. No. 47641-U) was purchased from Supelco, USA. Standard compounds *viz.*, benzoic acid, caffeic acid, gallic acid, (±)-catechin, trans-cinnamic acid, p-coumaric acid, cyanidin chloride, delphinidin chloride, 4-hydroxybenzoic acid, malvin chloride, malvidin chloride, naringin, pimaricin, quercetin, uracil, resveratrol, sinapic acid, salicylic acid, syringic acid, kaempferol and α-mangosteen were purchased from Sigma-Aldrich, USA. Apigenin, butylated hydroxyl toluene (BHT), ter-butylhydroquinone (TBHQ), lauryl gallate, luteolin, myricetin, nordihydroguaiaretic acid (NDGA), octyl gallate, propyl gallate, trans-ferulic and vanillic acid were procured from Fluka-Analytical, Sigma-Aldrich, USA. Vanillin was procured from Himedia, Mumbai, India. Rutin was procured from Ozone International, Mumbai, India. HPLC grade water, methanol and acetonitrile were procured from Ramkem, New, India. Sulphuric acid (HPLC grade) was procured from E. Merck, Germany.

HPLC System and operating conditions

Chromatographic separations were carried out on the Waters HPLC system, equipped with 1525 separation module, Rheodyne 7725i injector (loop capacity 20 µl) and 2996 photodiode array detector controlled by Empower software. The column was fitted with Discovery® C18 columns, 25 cm x 4.6 mm, 5 µm (Supelco analytical, Sigma-Aldirch, USA). Column temperature was maintained at 25 °C (temperature control module II, Waters). The experimental condition was fixed at 25±1 °C. The acidified water was prepared by gently dissolving 271.7µl of HPLC grade H_2SO_4 in packaged HPLC grade water and kept undisturbed for some hours for dispersion. Afterward the pH was checked and adjusted to 2.00±0.01 with dil. H_2SO_4.

Collection and processing of fruit sample

The fruit sample of *Garcinia xanthochymus* Hook. f. and *Averrhoa carambola* L. were collected from the Imphal-west District, Manipur. *Docynia indica* (Wall.) Decne. and *Rhus semialata* Murray were collected from Churachandpur District, Manipur and *Garcinia pedunculata* Roxb. from Bishnupur District, Manipur. Edible portion of each of the fruit was chopped and dried in an air circulating oven until a constant weight was obtained. Dried sample was ground into powdery form by using a kitchen blender and defatted by macerating in petroleum ether for 72 h.

Extraction of phenolics for HPLC analysis

Pulverized defatted sample was macerated in an extraction solvent (1:10 w/v) composed of methanol and 1 N H_2SO4 (4:1) for *ca.* 30 min. The synthetic antioxidant, 2 % (w/w) of BHT was added to the mixture to prevent any oxidative breakdown. The mixture was then sonicated (UltraSonic Cleaner, model No. 08895-22, Cole-Palmer, USA) at sonic speed 40, 65 °C for 1 h. The mixture was then centrifuged at 12,000 rpm for 15 min. The supernatant was syringed filtered through 0.22 µm PTFE filter and kept stored at -20 °C until analyses.

HPLC System calibration and validation by using the standard test mixture

Reversed phase test mix was used for the HPLC system calibration and validation for the analyses of phenolics. The test mixture components, HPLC system, parameters and conditions were given in **Figure 1**. This test mixture was tested in the specified HPLC system and column mentioned above at 25 °C.

RP-HPLC-PDA method for quantification of phenolic compounds

A gradient elution programme using acidified water (pH 2.0) (A) and acetonitrile (B) as organic modifier was chosen: 1) 0–2 min, 12 % B, flow rate (FR) 0.4ml/min, 2) 2–5 min, 12 % B, linear gradient from 0.4 to 0.5 ml/min, 3) 5–50 min, linear gradient from 12 to 50% B and 0.5 to 0.65 ml/min, 4) 50-55 min, linear gradient from 50% to 90% B and 0.65 to 0.8 ml/min, 5) 55-65 min, isocratic 90% B and FR from 0.8 to 0.7 ml/min and 6) Reconditioning phase: 65-70 min, linear gradient from 90 to 12% B and from 0.7 to 0.4 ml/min. Column and systems were regularly washed with methanol after three runs. 10 µl of appropriate concentration was injected to render a sensitive chromatogram whose

absorbance unit is less than 0.5 AU. **Figure 2** illustrates the simultaneous separation of standard mixture of 34 phenolic compounds at 6 different wavelengths. Benzoate derivatives like gallic acid, syringic acid, vanillic acid, vanillin, BHT, propyl gallate, octyl gallate, lauryl gallate, TBHQ, NDGA, 4-hydroxybenzoic acids and cinnamate derivatives like caffeic acid, trans-ferulic acid, sinapic acid, p-coumaric acid were monitored and quantified at 280 nm.

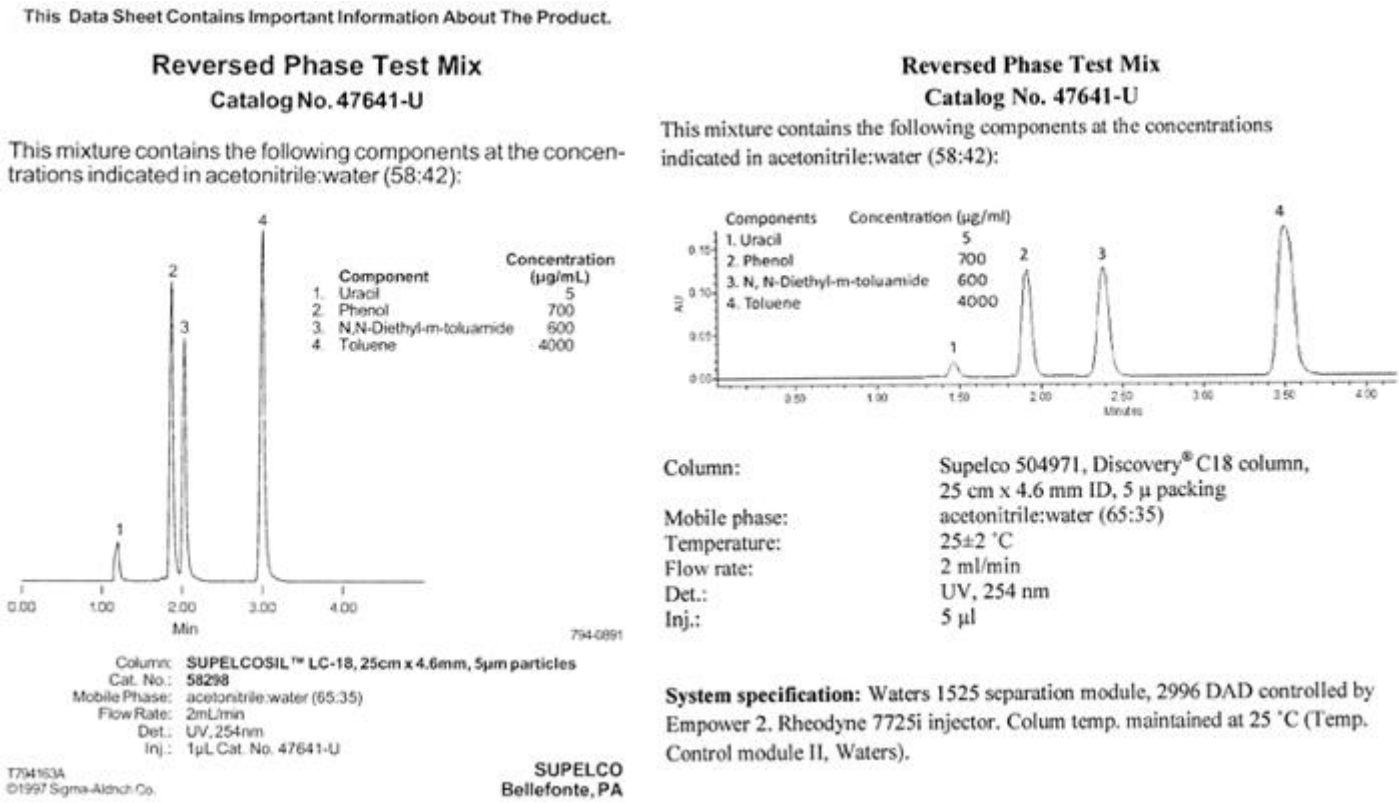

Figure 1 Comparative chromatograms of test mixture (Reversed phase test mix): Left panel-reference, Right panel-test evaluation

Flavanol like kaempferol, myricetin, quercetin and rutin were monitored and quantified at 370 nm. Flavonone like naringin, pimaricin, alpha mangosteen and flavones like apigenin, resveratrol and luteolin were monitored and quantified at 320 nm. Anthocyanidins groups like cyanidin, malvidin, delphinidin, and malvin were monitored and quantified at 510 nm. Others simple phenolic acids like salicylic acid and benzoic acid were quantified at lower wavelength, 210 nm. Internal response factor (IRF) for each standard compound was calculated with respect to specific internal standard used for 280 nm (NDGA), 320 nm (Luteolin), 370 nm (luteolin) and 510 nm (malvin) from the standard test mixture. Appropriate concentration of internal standards and void volume marker (uracil) was added to the sample extract. Identification and quantification of basic phenolic compounds in the fruit extract was then carried out by comparative evaluation of its sensitive peaks obtained at six different wavelengths (Figure 2). Where, *IS* = internal standard, ddw = defatted dry weight.

$$Internal\ Response\ factor\ (IRF) = \frac{Area_{IS} \times Conc._{sample}}{Conc._{IS} \times Area_{sample}}$$

$$Conc.of\ the\ sample\ (\mu g/ml) = \frac{Conc._{IS} \times Area_{sample} \times IRF}{Area_{IS}}$$

$$\%\ Content\ (mg/100g)of\ phenolic\ = \frac{Dilution\ factor \times Multiplier}{Wt.of\ the\ sample\ (g\ ddw) \times 100 \times Conc.of\ the\ sample\ (\mu g/ml)}$$

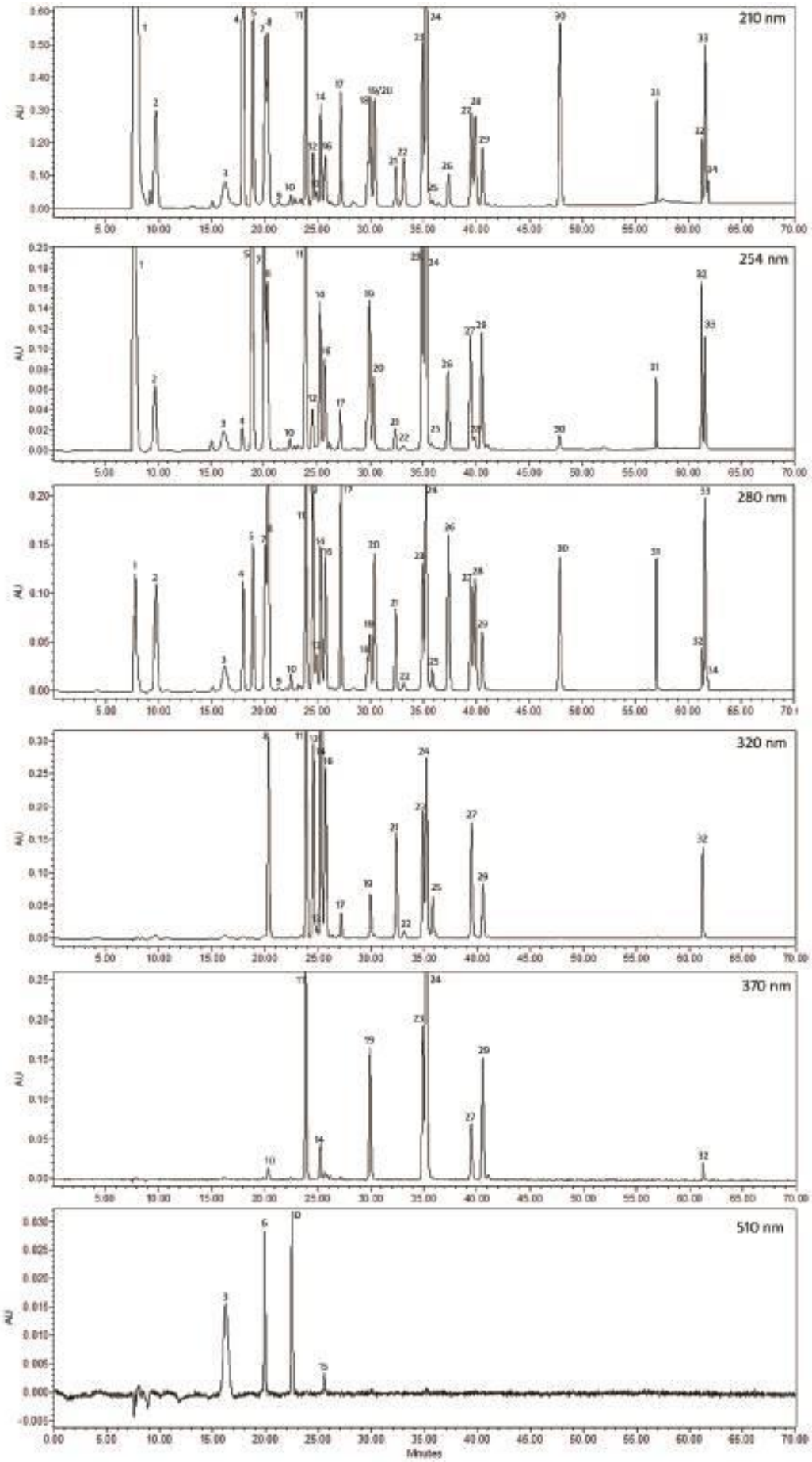

Figure 2 Chromatograms for simultaneous profiling of 34 standard phenolics mixture at six different wavelengths using PDA detector. Peak assignment: refer Table 3

Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD) of three independent determinations. Calculation of IRF, separation factor, and other calculations required in quantification were performed in MS excel 2007.

Results and Discussion

A broadly applied extraction procedure is solvent extraction using extractants such as methanol, ethanol and acetone or mixtures of these with water [10] for the recovery of a wide range of polyphenols of diverse phenolic structures [11]. Most phenolic derivatives present in the plant matrix are stored in vacuoles and are commonly extracted in alcohol or organic solvents [6]. Hydrolysis of ester to carboxylic acid has been one strategy employed to simplify the analysis and gain a more specific picture of the phenolic acid profile in foods [6]. Extracting solvent comprised 1 N H_2SO_4 and methanol in the ratio of 2:8 giving a final pH of 0.88. This extracting solvent mixture on treatment with ultrasonic hot water bath helped in de-esterification and breaking down of phenolic derivatives into simpler phenolic constituents simplifying profiling through HPLC methods. However, certain phenolic derivatives like sugar linked and complex phenolics are underestimated. Nonetheless, cleavage of ester bonds simplified the analysis by reducing matrix interferences and derivatives. Antioxidants such as *tert*-butylhydroquinone (BHA), 2, 6-di-*tert*-butyl- 4-methylphenol (BHT), ascorbic acid or sulphites are often added to the extraction solvent in order to protect the analytes from oxidation [12]. Similarly, in our phenolic extraction, synthetic antioxidant, BHT (2 % w/w) was added to minimize oxidation of phenolics to their respective quinones during the course of phenolic extraction. Synthetic antioxidant was chosen instead of natural antioxidant in order to avoid any peak inflation during HPLC analysis. Synthetic BHT was used as the representative molecule for the assessment of percentage recovery of phenolic components. Percentage recovery ranges from 84 to 104 % in the five different fruit extracts. Analysis of polyphenolic compounds is influenced by their chemical nature, the extraction method employed, particle size, storage conditions, assay method, selection of standards and presence of interfering substances such as waxes, fats, terpenes and chlorophylls [13]. Therefore, no complete satisfactory extraction procedure was found suitable for extraction of all phenolics or a specific class of phenolic substances in foods [13].

Any change in the column pressure other than program automatic changes was minimized by adjusting in the column thermostat and room air condition. These adjustments help to achieve precise and reproducible chromatograms. HPLC system was checked with reference to the standard reversed phase test mix by simulating the experimental and working conditions of the standard reference (Figure 1 left panel). The operating temperature of the standard reference was not given; however, for our experimental validation, 25 ⁰ C was fixed. The column type, dimension and specifications of the reference and our column are same. All the four components of the test mixture eluted in less than 4 min in both cases (*cf.* left and right panel of Figure 1). Sharp peaks were not observed in our system which may be partly due to operating temperature or larger load (5 µl) as compared to 1 µl of standard reference. Relative retention time of peak 2 and 3 was however found little higher than the corresponding reference. From the above experimental validation, no major deviation from the standard reference was found. Retention time, retention factor, separation factor and IRF of 34 standard compounds were given in **Table 1**. Uracil was taken as void volume marker based on retaining-ability in C18 column and subsequently retention factor of all standards were evaluated with reference to uracil. Retention factor is independent of some key variable factors, including small flow rate variation and column dimension. Separation factor or selectivity of all 34 standard compounds was found above 1.0 implying good separation. Chromatogram for each 210, 254, 280, 320, 370 and 510 nm were simultaneously recorded by using a PDA detector. Such method of simultaneous and multiple acquisitions help resolving ambiguous peaks and enhancing analytical sensitivity since different standards have certain sensitive wavelengths. Among six wavelengths, 280 nm displayed maximum peaks and therefore it was taken as main reference chromatogram while other chromatograms were not shown in **Figure 3.** Though, quantification was done based on the highest sensitivity from among six wavelengths. The order of elution of anthocyanidins: malvin > delphinidin > cyanidin > malvidin, hydroxycinnamates: caffeic > p- coumaric > sinapic > ferulic > trans-cinnamic acid, benzoates: gallic acid > vanillic acid > syringic acid, flavanols: rutin > myricetin > quercetin > kaemferol, and flavones: Luteolin > apigenin were found in concord with Kim and Lee (2002) [14]. However, elution orders of whole 34 compounds slightly differed from those of Kim and Lee (2002) [14]. The elution order of 34 compounds was found as uracil (void volume marker) > gallic acid > malvin > (±)-catechin > 4-hydroxy benzoic acid > delphinidin > syringic acid > vanillic acid > caffeic acid > cyanidin > rutin > vanillin > p-coumaric acid > sinapic acid > malvidin > trans-ferulic acid > naringin > benzoic acid > myricetin > propyl gallate > resveratrol > salicylic acid > luteolin > quercetin > pimaricin > trans-cinnamic acid > apigenin > TBHQ > kaempferol > NDGA > octyl gallate > α-mangosteen > lauryl gallate > BHT within 70 min.

Table 1 Retention time (t_R), retention factor (k), separation factor (α), concentration, internal response factor (IRF) and acquisition wavelength of standard phenolic compounds.

Peak no.	Standard compound	λ[a]	t_R[b]	k[c]	α[d]	Conc. (mg/l)	IRF[e]
1	Uracil (t_o)	280	7.64±0.08	-	-	122	5.20
2	Gallic acid	280	9.73±0.34	8.738	-	31.1	0.28
3	Malvin chloride	510	16.22±0.34	15.22	1.742	16.6	0.40
4	(±)-Catechin	280	17.96±0.28	16.96	1.114	97.7	1.67
5	4-OH-benzoic acid	280	18.89±0.20	17.89	1.055	62.2	0.69
6	Delphinidin	280	19.91±0.01	18.91	1.057	16.6	-
7	Syringic acid	280	20.05±0.24	19.06	1.008	31.1	0.39
8	Vanillic acid	280	20.31±0.23	19.31	1.013	44.4	0.22
9	Caffeic acid	280	21.36±0.15	20.36	1.055	31.1	16.06
10	Cyanidin	510	22.46±0.36	21.46	1.054	33.3	4.55
11	Rutin	370	23.89±0.17	22.89	1.067	42.2	0.23
12	Vanillin	280	24.56±0.02	23.56	1.029	26.6	0.23
13	p-coumaric acid	280	24.91±0.19	23.91	1.015	26.6	1.27
14	Sinapic acid	280	25.30±0.16	24.3	1.017	44.3	0.55
15	Malvidin	510	25.54±0.11	24.55	1.01	38.8	-
16	Ferulic acid	280	25.71±0.27	24.72	1.007	31.1	0.40
17	Naringin	280	27.18±0.17	26.19	1.059	62	0.66
18	Benzoic acid	280	29.73±0.37	28.73	1.097	62	3.33
19	Myricetin	370	29.93±0.04	28.94	1.007	15.1	0.47
20	Propyl gallate	280	30.35±0.11	29.35	1.014	31.1	0.38
21	Resveratrol	320	32.37±0.31	31.37	1.069	15.5	0.31
22	Salicylic acid	210	33.11±0.15	32.11	1.024	12.4	2.61
23	Luteolin	370	34.91±0.24	33.91	1.056	26.6	0.36
24	Quercetin	370	35.23±0.27	34.24	1.01	42	0.30
25	Pimaricin	320	35.82±0.13	34.83	1.017	9.2	0.73
26	Trans-Cinnamic	280	37.3450.13	36.35	1.044	15.5	0.14
27	Apigenin	320	39.46±0.07	38.47	1.058	13	0.19
28	TBHQ	370	39.85±0.28	38.85	1.01	10.6	3.06
29	Kaempferol	280	40.54±0.23	39.55	1.018	111	0.13
30	NDGA	280	47.85±0.10	46.86	1.185	88.6	1
31	Octyl gallate	280	56.99±0.29	56	1.195	31	0.83
32	α-mangosteen	320	61.27±0.02	60.27	1.076	32	2.07
33	Lauryl gallate	280	61.56±0.02	60.57	1.005	62	0.81
34	BHT	280	61.81±0.02	60.81	1.004	15.5	3.22

λ[a]: wavelength (nm) used in quantification. t_R[b]: Retention time given in mean ± standard deviation of three independent analysis. k[c]: Retention factor = $(t_R - t_o)/t_o$, where t_R and t_o denotes retention time of sample and uracil (unretained compound) respectively. α[d]: separation factor = k2/k1, where k1 and k2 represent retention factor of neighbouring peaks. IRF[e]: Internal response factor *w. r. t.* IS (NDGA).

The composition of polyphenolic phytochemical is influenced by maturity, cultivar cultural practices, geographic origin, climatic conditions, storage conditions, and processing procedures [15]. For the present analysis, the fruit samples were harvested at their appropriate maturity stage. Plants of Clusiaceae family are rich sources of xanthones, biflavonoids and benzophenones like garcinol [16]. In *Garcinia,* depending on the originating tree, the resin composition changes, but in all cases it contains about 70 % of organic constituents that are insoluble in water [17]. In *G. xanthochymus,* yellow insoluble resins were substantially found which were first washed out by petroleum ether before phenolic extraction. Henceforth, a substantial amount of compounds like xanthones, benzophenones etc. were probably excluded in our present analysis. Defating treatment rendered good resolution of almost whole peaks (Figure 3 A). In *G. xanthochymus*, 10 phenolic compounds *viz.,* gallic acid, syringic acid, vanillic acid, caffeic acid, ferulic acid, myricetin, propyl gallate, trans-cinnamic acid, octyl gallate and lauryl gallate were quantified. However, many peaks were left unidentified due to lack of standard compounds. Highest content was caffeic acid (2157.63 mg/100g ddw) followed by myricetin (367.37 mg/100g ddw) and gallic acid (281.91 mg/100g ddw) (**Table 2**). Complex derivatives of benzophenones, biflavonoids, xanthones and triterpenes were not able to identify due to lack of appropriate standards as well as partly due to different extraction procedure employed.

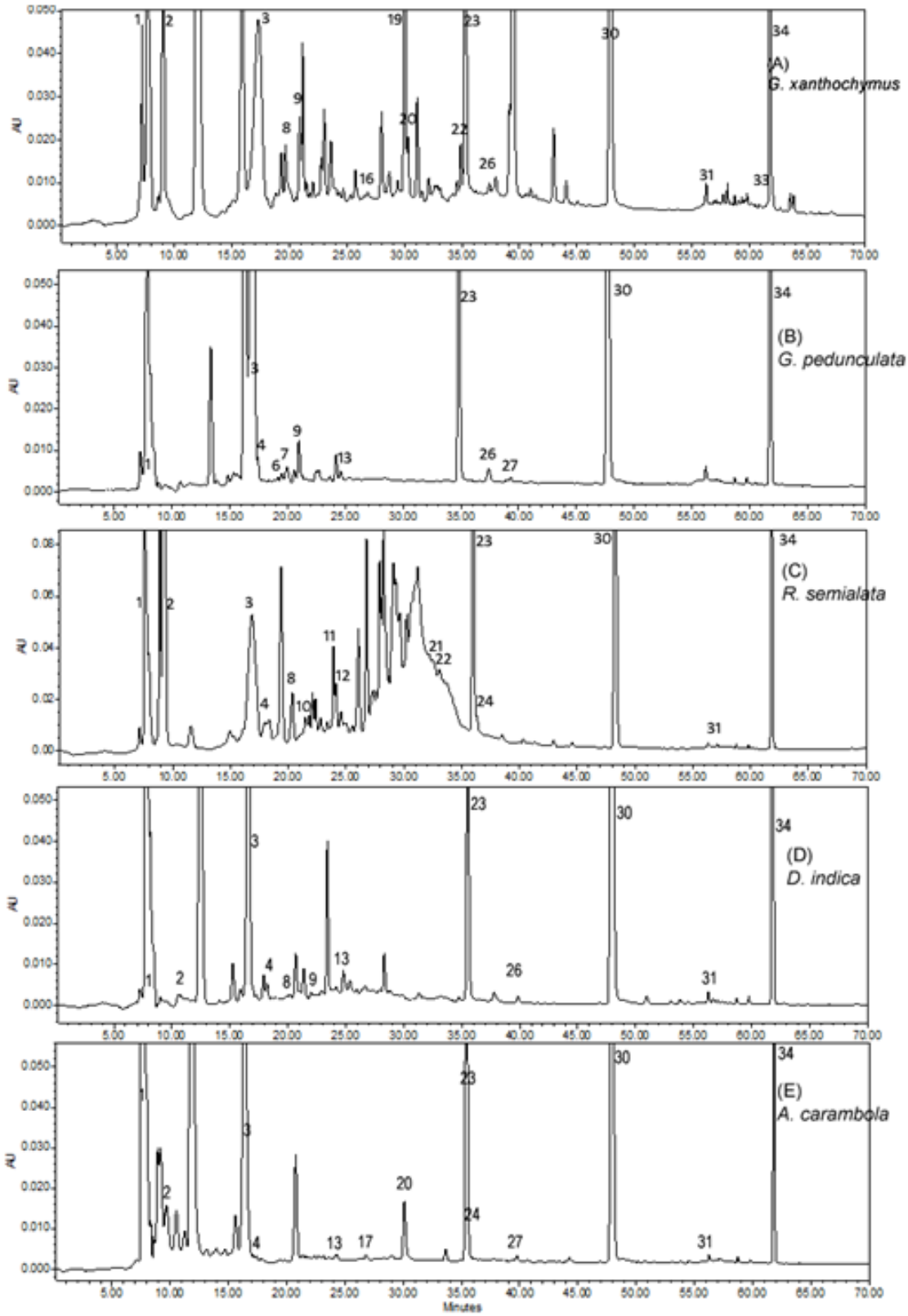

Figure 3 Phenolic profile (chromatograms at 280 nm) of five wild fruit: (A) *G. xanthochymus*, (B) *G. pedunculata*, (C) *R. semialata*, (D) *D. indica and* (E) *A. carambola.* Peak assignment: refer Table 3

In *G. pedunculata,* 8 phenolic compounds *viz.*, catechin, delphinidin, syringic acid, caffeic acid, p-coumaric acid, trans-cinnamic acid, apigenin, and α-mangosteen were quantified. Highest content was caffeic acid (391.52 mg/100g ddw) followed by catechin (15.66 mg/100g ddw) and delphinidin (15.08 mg/100g ddw) (Table 2). Alpha-mangosteen (xanthone derivative) was able to quantify (1.48 mg/ 100g ddw) in *G. pedunculata* but not in *G. xanthochymus*. Presence of *p*-coumaric acid suggests the presence of *p*-coumaric derivatives. Apigenin was also detected that may be residue of some flavones derivatives.

Table 2 Comparative phenolic content of the five wild fruits as quantified by HPLC-PDA method

Compounds	***G. xanthochymus*** **(mg/100g ddw)**	***G. pedunculata*** **(mg/100g ddw)**	***R. semialata*** **(mg/100g ddw)**	***D. indica*** **(mg/100g ddw)**	***A. carambola*** **(mg/100gddw)**
Gallic acid	281.91±10.71	-	823.34±34.85	1.37±0.45	3.26±0.34
Catechin	-	15.66±0.65	115.39±4.87	11.15±0.58	28.67±0.75
Delphinidin	-	15.08±0.75	-	-	-
Syringic acid	39.27±1.64	4.58±0.19	-	-	-
Vanillic aicd	37.74±1.58	-	26.34±1.11	0.673±0.32	-
Caffeic acid	2157.63±97.85	391.52±16.33	-	241.35±12.54	-
Cyanidin	-	-	142.19±5.53	-	-
Rutin	-	-	28.84±1.22	-	-
Vanillin	-	-	10.7±0.45	-	-
p-Coumaric acid	-	6.15±0.25	-	15.21±0.79	35.63±1.16
Ferulic acid	3.92±0.16	-	-	-	-
Naringin	-	-	-	-	19.28±1.49
Myricetin	367.37±15.42	-	-	-	-
Propylgallate	28.84±1.20	-	-	-	166.03±8.35
Resveratrol	-	-	32.59±1.37	-	-
Salicylic acid	-	-	631.14±26.71	-	-
Quercetin	-	-	17.94±1.59	-	3.26±0.34
T-Cinnamic acid	4.35±0.18	1.48±0.06	-	0.917±0.04	-
Apigenin	-	2.09±0.67	-	-	20.73±1.08
Kaempferol	-	-	-	47.13±2.02	-
Octyl gallate	22.55±0.94	-	1.89±0.08	3.41±0.55	25.27±0.92
α-Mangosteen	-	2.48±0.71	-	-	-
Lauryl gallate	5.13±0.21	-	-	-	-

There are many reports of various bioactive compounds identified in *R. semialata,* like semialatic acid, identified as 3α-hydroxy-3β, 19-epoxydammara-20, 24*E*-dien-26-oic acid [18]. Due to lack of standards, such compounds were unable to authenticate in our present study. In the fruit of *R. semialata,* 10 basic phenolic compounds viz., gallic acid, catechin, vanillic acid, cyanidin, rutin, vanillin, resveratrol, salicylic acid, quercetin, and octyl gallate were quantified. Highest content was gallic acid (823.34 mg/100g ddw) followed by salicylic acid (631.14 mg/100g ddw) and cyanidin (142.19 mg/100g ddw) (Table 2). 6-pentadecylsalicylic acid, an anti-inflammatory like other salicylates was first reported by Arthur (1954) [19] and Kuo et al. (1991) [20] in *R. semialata*. Later, such salicylic derivative was known by anacardic acid (AA). Chemically, AA is a mixture of several closely related organic compounds each consisting of a salicylic acid substituted with saturated or unsaturated alkyl chain that has 15–17 carbon [21]. These AA possessed bactericide, fungicide, insecticide, anti-termite and molluscicide properties and acts as a therapeutic agent in the treatment of pathophysiological disorders such as cancer, oxidative damage, inflammation and obesity [22]. In our analysis, salicylate was found abundantly (631.14 mg/100g ddw) after gallate suggesting the significant amount of various types of AAs. Catechin and cyanidin was quantified at about *ca.* 100 mg/100g ddw. A Prominent unit of tannins *i.e.*, gallic acid was found highest (823.34 mg/100g ddw) suggesting numerous types of tannins (galloyl ester derivatives) present in the fruit. Catechin based tannins were also likely to present in the fruit as catechin was found in the analysis. The resolution of various components eluted in between 25-35 min was poor, which was probably due to the complex interaction of fatty components with phenolic compounds (Figure 3 C).

In the fruit of *D. indica,* 8 phenolic compounds *viz.*, gallic acid, catechin, vanillic acid, caffeic acid, p-coumaric acid, trans-cinnamic acid, kaempferol, and octyl gallate were quantified. Highest content was caffeic acid (241.35 mg/100g ddw) followed by kaempferol (47.13 mg/100g ddw) and p-coumaric acid (15.21 mg/100g ddw) (Table 2).

In the fruit of *A. carambola*, 8 phenolic compounds *viz.*, gallic acid, catechin, p-coumaric acid, naringin, propyl gallate, quercetin, apigenin, and octyl gallate were quantified. Highest content was propyl gallate (166.03 mg/100g ddw) followed by p- coumaric acid (35.63 mg/100g ddw) and delphinidin (28.67 mg/100g ddw) (Table 2). It was reported by Shui and Leong (2004) [23] that proanthocyanidins formed by catechin and epicatechin, gallic acid in gallotannin forms were the significant contributor of antioxidant activity of the fruit. O-glycosylated flavonoids: quercetin-3-β-d-glucoside and rutin were reported to present in the fruit by Tiwari et al. (1979) [24]. The fruit sample after hydrolysis revealed the presence of quercetin (3.26 mg/100g ddw); however, rutin was not able to identify in our study. Apigenin (20.73 mg/100g) was found, however, cyanidin was not able to quantify in our study.

Conclusions

RP-HPLC-PDA method and optimized gradient program using aqueous phase (pH 2.0) and acetonitrile for simultaneous profiling of phenolic compounds was effectively applied to profile phenolic components of five different fruit samples. In our present analysis, 34 standard compounds were used and simultaneously profiled at 6 different wavelengths *viz.*, 210, 254, 280, 320, 370 and 510 nm using a PDA detector. The method has enabled to distinguish ambiguous peaks by comparing 6 chromatograms according to their photosensitive wavelengths. Extraction of phenolics from defatted dried sample greatly reduces the lipid interferences during HPLC analysis. In each fruit sample, 8 to 10 basic phenolic compounds were quantified and various allied compounds were detected. However, several peaks remain unidentified due to lack of standards. The proposed RP-HPLC-PDA gradient program is a cost effective method for profiling and quantification of phenolic compositional units that could be applied to various types of sample.

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