IJFANS
International Journal of Food And Nutritional Sciences

Volume 5, Issue 3, July 2016, www.ijfans.com e-ISSN: 2320-7876

# INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

## IMPACT FACTOR ~ 1.021

Official Journal of IIFANS

IJFANS International Journal of Food and Nutritional Science

*e-ISSN 2320 -7876 www.ijfans.com*
*Vol. 5, No. 3, July 2016*


***Research Paper*** ***Open Access***

# EXTRACTION, IDENTIFICATION AND ANTIOXIDANT PROPERTIES OF BIOACTIVE COMPONENTS OF AMLA (EMBLICA OFFICINALIS) POMACE POWDER

Poonam Mishra[1]* and Charu Lata Mahanta[1]

*Corresponding Author: Poonam Mishra, ✉ poonam@tezu.ernet.in

Received on: 9th February, 2016 Accepted on: 26th March, 2016

Amla has been traditionally used as an important herbal medicine in southeast Asia and has been known for its potent antioxidant activity. However, amla pomace has never been investigated for its phenolic content and antioxidant properties. In this study, the dry pomace powder of amla fruit was extracted with ethanol water mixture, and then the extracted powder was partitioned by diethyl ether, butenol and Ethyl Acetate (EA). The EA fraction was purified by Sephadex column chromatography and preparatory HPLC. The ethyl acetate, butanol, and aqueous fractions obtained after column chromatography were analyzed for their phenolic content and diphenyl picryl hydrazil free radical (DPPH*) scavenging activity. Major polyphenols contained in ethyl acetate fractions of pomace powder were gallic acid, catechin, caffeic acid and syringic acid. Three isolated components from pomace powder were identified as hydroxytyrosol (E-1), gallic acid (E-2) and catechin (E-3) by using analytical HPLC and GC-MS. All the compounds exhibited significant DPPH* scavenging activity. Gallic acid exhibited the highest DPPH* scavenging activity.

***Keywords:*** Amla pomace powder; Gallic acid, Hydroxytyrosol, Catechin, DPPH

## INTRODUCTION

Oxidative stress, induced by oxygen radicals is one of the main causative factors of various degenerative diseases, such as cancer (Muramatsu *et al.*, 1995), atherosclerosis (Steinberg *et al.*, 1998), gastric ulcer (Das *et al.*, 1997), and other conditions (Oliver *et al.*, 1987; and Smith *et al.*, 1996).

The strong antioxidant activity of the phenolics and their ability to defend cells against oxidative damage because of the free radicals are well established (Kahkonen *et al.*, 1999). Conjugated ring structures of the hydroxyl group of many phenolic compounds has the potential to function as singlet oxygen (Husain *et al.*, 1987) and as free radical scavenger by scavenging superoxide anions (Robak and Dryglewski, 1988) and lipid peroxy radicals (Torel *et al.*, 1986).

Phenolics are the major chemical constituents of Amla (Kusirisin *et al.*, 2009) and these substances have strong antioxidant property and might contribute to the healthy affects of Amla. Several active consituents like ellagic acid, gallic acid, chebulininc acid, chebulagic acid, etc. have been extracted from the aqueous extract of Amla (Zhang *et al.*, 2003; E1-Deousky *et al.*, 2008; and Luo *et al.*, 2009 and 2011). Amla pomace is a major processing waste of Amla juice industries has never been investigated for its bioactive properties. The objective of present investigation is to characterize the polyphenols present in Amla pomace by analytical HPLC, GC-MS and FTIR.

## MATERIALS AND METHODS

### Raw Material

*Chakaiya* variety of Amla was procured from local market

[1] Department of Food Engineering & Technology, Tezpur University, Napaam, Assam- 84028, India.

of Allahabad, India. Grated Amla shreds were pulped in laboratory mixer and the juice was extracted by using double fold muslin cloth. Pomace left after the extraction of the juice was dried at 40 °C and ground into powder in a laboratory mill by passing 0.5 mm sieve. The pomace powder was stored at refrigerated temperature for further extraction and purification of phenolics. On an average 0.58±0.02 kg of pomace powder was obtained from 5 kg of fresh Amla fruit.

## Reagents

Reagents and solvents were purchased from Merck and standards of phenolics and DMDCS (dimethyl dichloro silane) were procured from Sigma eldrich.

## Purification and Extraction of Total Phenolics from Amla Pomace Powder

The phenolics in 30 g of Amla pomace powder was extracted (under optimised conditions of extraction from Amla powder as reported in Chapter-3) in 300 mL of ethanol (78%) by shaking in an incubator shaker for 4 h at 30.50 °C and 4.5 pH. The extract was centrifuged in refrigerated centrifuge at 4 °C and 8000 rpm for 15 min and supernatant was collected. The residue was re-extracted and then further centrifuged. Both the collected supernatants were pooled and evaporated under reduced pressure followed by freeze drying under vacuum. Ethanolic extract of pomace powder (8.3 g) was dispersed in 40 mL of water and was partitioned with ethyl ether (100 mLx3), ethyl acetate (100 mLx3) and butanol (100 mLx3) successively. All four fractions were evaporated separately under reduced pressure at 45 °C for ethyl acetate and ethyl ether fraction and at 65 °C for butanol and aqueous fraction as per the method of Liu *et al.* (2012) and freeze dried under vacuum. Ethyl acetate extract powder was light golden yellow color while butanolic extract powder was of light reddish yellow in color. The recovery of ethyl acetate, ethyl ether, butanol and aqueous fractions were, 4.83 g, 0.25 g, 3.45 g and 2.50 g, respectively. Ethyl acetate, butanol, aqueous and ethyl ether fractions were analyzed for their TPC content and % DPPH* scavenging activity. As ethyl acetate fraction exhibited highest DPPH* scavenging activity, it was further purified by fractionation on a Sepahdex G-25 (25-100 µm, Sigma Aldrich) column (450 x 25 mm) with water/methanol (100:0-0:100) as the eluent and obtained six different fractions (I-VI). All six fractions were evaluated for characterization of the phenolics present by HPLC and FTIR.

## Fourier Transform Infrared Spectroscopy (FTIR)

All extracted powders and different fractions obtained after column chromatography were scanned from 4000 to 650 $cm^{-1}$ with a resolution of 4/$cm^{-1}$. FTIR spectra was collected at room temperature by coupling ATR accessory to an FTIR spectrometer (Perkin Elmer, Spectrum 100). The time required to complete background was 20 s. Average of 3 scans were collected (Mayachiew and Devahastin, 2010).

## Total Phenolic Content (TPC)

Estimation of TPC was performed by both Folin-Ciocalteu Reagent (FCR) method and HPLC method. Estimation of FCR was conducted by the method of Liu *et al.* (2012). For estimation of TPC of extracted powder, 50 mg of extracted powder was mixed with 5 mL of methanol and the mixture was stirred for 30 min at 25 °C. The stirred mixture was centrifuged and supernatant was analysed for its total phenolic content as the same method given by Liu *et al.* (2012).

Analytical HPLC was conducted on a Waters (Breeze-2) liquid chromatography fitted with a C-18, reversed phase (5 µm) column (7.2 x 300 mm) following the method of Seruga *et al.* (2011) with slight modifications. In short, orthophosphoric acid (0.1%) was taken as solvent A and 100% HPLC grade methanol was used as solvent B for the separation of phenolics. The elution conditions used for the identification of phenolics were as follows: 0-30 min from 5% B to 80% B; 30-33 min 80% B; 33-35 min from 80% B to 5% B; 35-40 min at 5% B; flow rate 0.8 mL/min. Column temperature and injection volume were kept constant at 20 °C and 20 µL, respectively. The standards of gallic acid, chlorogenic acid, syringic acid, catechin, p-coumaric acid, and quercetin were estimated at two different wavelengths, i.e., at 280 nm and 360 nm. Total area under curve at 280 nm was calculated as total phenolic content of the analyzed sample.

For identification of isolated components, 0.1 mg of the sample was dissolved in one mL of HPLC grade methanol and 20 µL of the sample was injected in HPLC with above prescribed method and obtained peaks were compared with the peak of standard components.

## 2, 6 Diphenyl Picryl Hydrazil Free Radical (DPPH*) Scavenging Activity

The DPPH* scavenging activity of extract of powder was estimated by the method of Luo *et al.* (2009) with slight

modifications. Briefly, 50 mg of the extracted powder were dissolved in 5 mL of methanol solution and shaken in an incubator shaker at 150 rpm at 25 °C for 30 min. 2 mL of filtered mixture of methanolic extract was mixed with 2 mL of methanolic solution containing 0.1 mM DPPH. The reaction mixture was mixed vigorously and then kept in the dark for 30 min. The OD was taken at 517 nm. The OD of control was measured by replacing the sample with methanol.

$$DPPH * Scavenging\ activity(\%) = \frac{(1 - absorbance\ of\ sample)}{absorbance\ of\ control} \times 100$$

### Assay of ABTS$^{+}$ Scavenging Activity

Antioxidant activity of extracted powder from Amla pomace was measured by the method of Cai *et al.* (2004), Re *et al.* (1999) and Wang *et al.* (2007).

### Preparatory HPLC

Fraction V had maximum DPPH* scavenging activity but recovery was very less hence, fraction III the second highest DPPH* radical scavenging activity was selected for further purification. Fraction III (170 mg) was further purified by reverse-phase HPLC (Waters 600E, Breeze-2, Milford, USA) on a $C_{18}$ l-Bondapak column (300 x 7.8 mm, flow rate = 1.5 mL/min) with methanol/water (40:60) for 40 min to yield pure compounds E1-E3.

### UV-Vis Spectrophotometric Analysis

Each of isolated compounds, E1-E3 (1 mg) was mixed with 10 mL of methanol and scanned from 200 to 750 nm, using double beam UV visible spectrophotometer (Evolution, Thermofisher, USA).

### Derivatization for GC-MS

The derivatization and identification by GC-MS was carried out by the method given by Proestos *et al.* (2006) with slight modification. For the silylation procedure, 100 µL of BSTFA was added in 0.01 mg of sample and vortexed in screw-cap glass tubes (previously deactivated with 5% dimethyldichlorosilane in toluene, and rinsed with toluene and then with methanol), and kept in a water bath, at 80 °C for 45 min. The silylated samples were injected into a GC-MS system of Perkin Elmer, model Clarus 600 gas chromatograph coupled 60 °C mass spectrometer and the mass range scanned at m/z 25-500. Elite 5 (30 m x 0.32 mm) was used as capillary column for the identification of phenolics. The detector and injector were set at 290 °C and 280 °C respectively. GC was performed in the split mode. The temperature programme was as follows : from 70° to 135 °C at 10 °C/min, from 135° to 220 °C at 15 °C/min, from 220° to 270 °C at 10 °C/ min and then held for 10 min. Rate of flow of carrier gas (helium) was kept constant at 1.9 mL/min. Identification of compound was achieved by comparing the spectral data obtained from the NIST libraries.

### Statistical Analysis

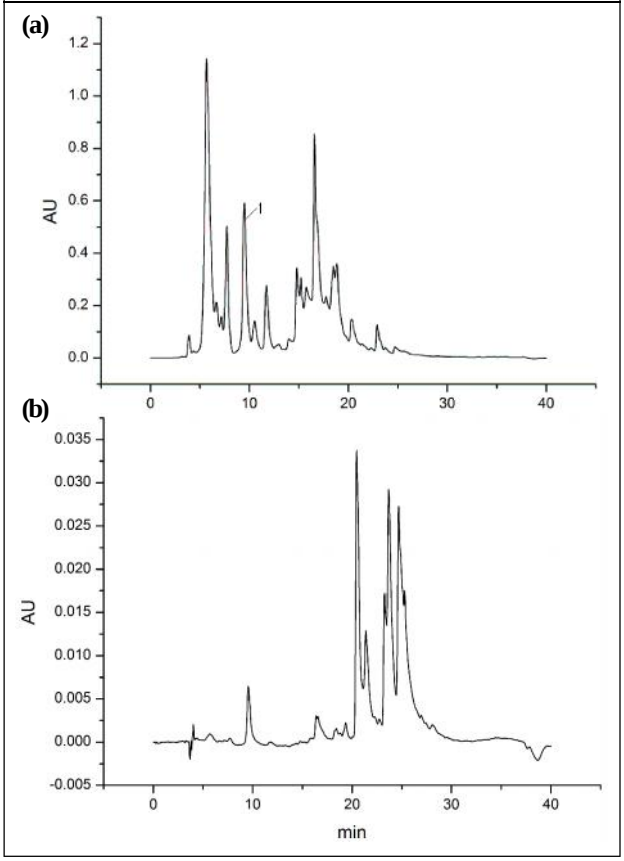
All experiments were performed in duplicate and the mean value and standard deviation were calculated using Excel version of 2003.

## RESULTS AND DISCUSSION

### Extraction of Phenolics Content

**Fig 1** presents the HPLC chromatogram of Amla pomace powder at (a) 280 nm and (b) 360 nm which show the presence of significant amount of total phenolic content in pomace powder that were not extracted in juice. Some amount of gallic acid was found to be available in the pomace. The total phenolic content in Amla pomace powder was 95 mg/g and gallic acid was 9.77 mg/g (**Fig 1a).**

**Figure 1: HPLC Chromatogram of Amla Pomace Powder (a) at 280 nm, and (b) at 360 nm (1) Gallic acid**



Ethanol extracted Amla pomace powder was dissolved in water and then further partitioned with ethyl ether, ethyl acetate and butanol. Diethyl ether fraction showed very poor scavenging activity and therefore was not taken for further studies. The present finding was in accordance with the observation of Roby *et al.* (2013) that diethyl ether had poor ability in extracting the phenolic compounds.

**Table 1: Bioactive Component and DPPH* Scavenging Activity of Aqueous, Ethyl Acetate, and Butanol Fractions of Amla Pomace Powder**

| Particular | Aqueous Fraction | Ethyl Acetate Fraction | Butanol Fraction |
|---|---|---|---|
| % Yield | 22.66+1.6[a] | 43.78+1.5[b] | 31.27+1.8[c] |
| % DPPH* scavenging activity/mg of powder | 4.55+0.3[a] | 14.29+0.1[b] | 8.85+0.2[c] |
| TPC by FCR (mg/g of GAE) | 121.32+2.7[a] | 389.65+2.2[b] | 264.33+2.0[c] |
| TPC by HPLC (mg/g) GAE | 43.20+0.4[a] | 98.80+0.2[b] | 119.50+0.3[c] |
| Gallic acid (mg/g) | ND | 8.81±0.3 | ND |
| Catechin (mg/g) | ND | 9.70+0.2 | ND |
| Caffeic acid (mg/g) | ND | 0.52±0.1 | ND |
| Quercetin (mg/g) | ND | ND | ND |
| p-Coumaric acid (mg/g) | ND | ND | ND |
| Syringic acid (mg/g) | ND | 3.60±0.1 | 15.60±0.2 |
| Vanillic acid (mg/g) | ND | ND | 3.74+0.2 |
| Chlorogenic acid (mg/g) | ND | ND | ND |

**Note:** Mean + standard deviation; Observation with different superscript in the same row differs significantly at 5% probability level.

**Figure 2: HPLC Chromatograms of (a) Aqueous Fraction, (b) Ethyl Acetate Fraction, and (c) Butanol Fraction, (1) Unidentified, (2) Gallic Acid, (3) Catechin, (4) Syringic Acid, (5) Caffeic Acid, (6) Vanillic Acid, and (7) Syringic Acid**

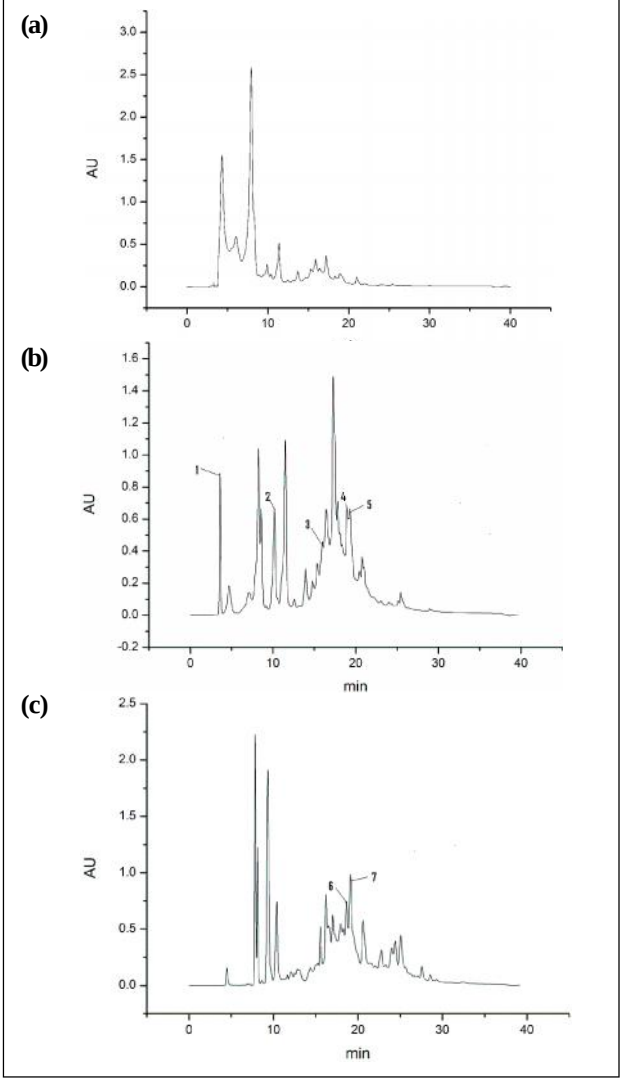



Table 1 presents the bioactive properties of ethyl acetate, butanol and aqueous fractions. The amount of total phenolic content varied in different extracts and ranged from 43.20 to 119.50 mg GAE/g of extracted powder (Table 1). One mg of ethyl acetate fraction showed 14.29% DPPH* scavenging activity, whereas butanol and aqueous fractions had 8.85% and 4.55% DPPH* radical scavenging activities, respectively for the same amount of the powder (Table 1). The less potency of free radical scavenging activity of aqueous fraction of Amla pomace powder is also corroborated by the low amount of TPC as estimated by the

HPLC method (Figure 2a) and FCR method (Table 1). The aqueous fraction had 43.20 mg/g GAE equivalent by HPLC method (Figure 2a) and 121.32 mg/g GAE equivalent by FCR method (Table 1). On the other hand, ethyl acetate fraction of Amla pomace powder had 98.80 mg/g GAE equivalent by HPLC method (Figure 2b) and 389.65mg/g GAE equivalent by FCR method (Table 1) and butanol fraction had 119.50 mg/g GAE equivalent by HPLC method (Figure 2c) and 264.33 mg/g GAE equivalent by FCR method (Table 1).

When analysed by HPLC, butanolic fraction of Amla pomace powder had significantly higher amount of phenolic content than the ethyl acetate fraction and aqueous fraction but the reverse trend was observed in case of FCR method. FCR method used for determination of total phenolic content gave considerably high value of phenolics than determined by HPLC method (Table 1). FCR method was not found suitable for the total phenolics estimation because the FCR reagent reacts not only with phenolics but also with a number of non-phenolic reducing compounds because of which the total phenolic content is overestimated (Ikawa *et al.*, 2003). Different phenolics can also react differently with the Folin-Ciocalteu's reagent, which results in lower absorption that underestimates the concentration of various compounds (Vinson *et al.*, 2002).

Inspite of having high amount of total phenolic content as determined by HPLC, the DPPH* scavenging activity of the butanolic extracts was comparatively lower than ethyl acetate extract fraction. One probable reason may be the high boiling point of butanol. The extract was exposed to higher temperature to remove the solvent which may adversely affect the availability of functional groups to form complexes with phosphomolybdate and hence butanolic extract showed less % DPPH* radical scavenging activity.

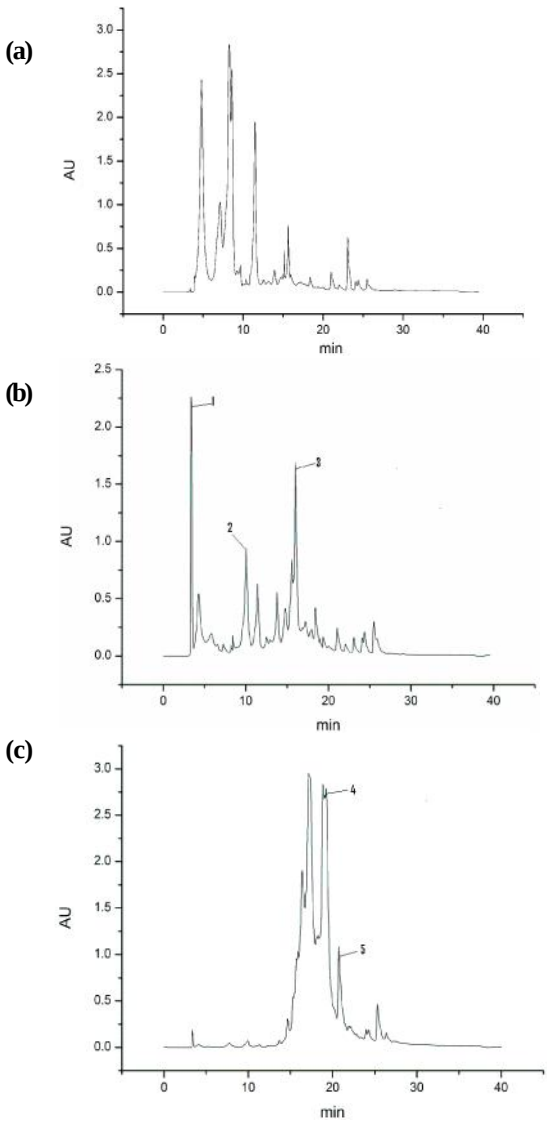
The low DPPH* scavenging activity of the aqueous extract of Amla pomace powder may be because of the phenolics left behind in the pomace after juice extraction were either present in bound form or had better affinity to solvents like ethyl acetate and butanol than water. So, during partitioning higher fractions of the phenolics went either in the ethyl acetate or butanol fraction. The present results suggested that the phenolic compounds in ethyl acetate fraction had maximum DPPH* radical scavenging activity and therefore ethyl acetate fraction was further purified and identified.

## Identification of Phenolics

### Reverse Phase High Performance Liquid Chromatography (HPLC)

Amla fruit is known to contain a range of active constituents but the availability of phenolic constituents in Amla pomace powder has never been investigated. Therefore ethyl acetate extract, butanolic extract, aqueous extract and the different fractions of Amla pomace powder obtained after column chromatography of ethyl acetate fraction were qualitatively and quantitatively analysed by reverse phase HPLC. The bioactive properties of HPLC chromatograms of above three fractions are given in Figure 2. Ethyl acetate fraction gave maximum recovery, i.e., 43.78% (of initial amount of ethanol extracted powder taken for the partition) whereas diethyl fraction powder gave the lowest recovery. The present findings were consistents with the findings of Liu *et al.* (2012). Table 1 shows that the major polyphenols contained in ethyl acetate fractions were gallic acid (8.81 mg/g, rt 10.09), catechin (9.70 mg/g, rt 15.94 min), caffeic acid (rt 19.48 min, 0.52 mg/g) and syringic acid (3.6 mg/g, rt 19.01). Butanol fraction had syringic acid (15.6 mg/g, rt 19.01,) and vanillic acid (3.74 mg/g, rt 18.51 ). The main phenolic compounds in ethyl acetate fraction of pomace powder were catechin (9.70 mg/g) and gallic acid (8.81 mg/g) (Table 1). p-Coumaric acid and quercetin could not be detected in any of the three fractions tested. Presence of major portion of the phenolics in ethyl acetate fraction confirms the finding of Liu *et al.* (2012) that the compounds with relatively high DPPH* scavenging activity and phenolics are present in ethyl acetate fraction. Six different ethyl acetate fractions obtained after column chromatography were further lyophilized before analysis. The % recovery of all six fractions (in respect of initial weight of sample taken for Sephadex column chromatography) i.e., I, II, III, IV, V and VI were 20.59%, 40.37%, 27.04%, 12.20%, 1.48% and 1.09% respectively (Table 2). It could be observed from the results that the highest extract yield was obtained in fraction II followed by III, I and IV, V and VI. Inspite of having good recovery of total phenolics in fraction II, the DPPH* scavenging activity of fraction II was significantly less than fraction III, IV and V suggesting the low availability of compounds having free radical scavenging activity low in that fraction. Fraction V showed exceptionally good free radical scavenging activity with a mean value of 149.86% but due to only 1.48% of recovery of powder it may not be economical for the extraction of the phenolics. Figure 3 presents the HPLC chromatograms of fraction III, IV and V of pomace powder.

**Figure 3: HPLC Chromatograms of (a) Fraction II, (b) Fraction III, and (c) Fraction IV, (1) Unidentified, (2) Gallic Acid, (3) Catechin, (4) Syringic Acid, and (5) Caffeic Acid**




It was found that catechin with 424.92 mg GAE/g and gallic acid with 17.15 mg GAE/g concentration were eluted in fraction III (Table 2 and Figure 3b) and this may explain the potency of this fraction to scavenge the DPPH*. Syringic acid (18.95 mg/g) and caffeic acid (10.19 mg/g) (Figure 3c) were eluted in fraction IV (Table 2) whereas 4.24 mg/g of syringic acid was eluted in fraction V. The high value of % DPPH* scavenging activity of fraction V indicates the presence of some phenolic compounds which have not been identified.

## FTIR Analysis

Figure 4 presents the FTIR spectra of different fractions of Amla pomace powder. It could be seen from Fig 4 that peak intensity of aqueous fraction was less than ethyl acetate and butanol fractions. Significant drop in functional groups were also observed in aqueous fraction which may explain the low DPPH* scavenging activity of the aqueous fraction Noticeably the presence of gallic acid peak at 3377, 1703, 1617, 1539 and 1254 $cm^{-1}$, 1100 $cm^{-1}$ and 1025 $cm^{-1}$ (Nirmaladevi *et al.*, 2010) were observed in ethyl acetate fraction at slightly different locations and confirms the presence of gallic acid in ethyl acetate fraction of Amla pomace powder. Significant drop in functional groups of fraction I was observed from Figure 4 whereas fraction V showed comparatively higher peak intensity of the functional groups which is substantiated by the potent ability of the fraction V to scavenge DPPH*. FTIR spectra also suggests the presence of some other phenolic compounds not yet detected in the present study. However, as the recovery of fraction V is very low it cannot be recommended for further purification or extraction.

## Detection of Isolated Compounds

Fraction V showed maximum DPPH* scavenging activity but its yield was low. On the other hand, fraction II with high yield showed poor DPPH* scavenging activity. Hence fraction III with moderate yield and moderate DPPH* scavenging activity was further purified by preparatory HPLC and three different components were isolated.

Isolated components, i.e., E-1, E-2 and E-3 had maximum absorbance at 275 nm, 275 nm and 277 nm, respectively. Representative chromatograms of isolated components are shown in Figure 5. By comparing HPLC chromatograms of E-2 and E-3 with standards it was observed that isolated component E-2 was gallic acid whereas E-3 was catechin. Oliver *et al.* (2010) had also reported the maximum absorbance of catechin is 278 nm.

The present finding also supported the findings of Luo *et al.* (2009) for the presence of gallic acid in Amla. Due to nonavailability of the standards, the component of E-1 could not be identified by HPLC. E-1 was identified through silylation process, which is an ideal procedure for analysis of thermolabile and non-volatile compound through GC-MS. By comparing the mass spectra of E-1 by NIST library the compound was detected as hydroxytyrosol. The molecular weight (m/Z) of TMS derivative of hydroxyl tyrosol was 370 and major characteristic fragments of

**Table 2: Characterization of Phenolics and DPPH* Scavenging Activity of Different Fractions Obtained After Column Chromatography**

| Particular | I | II | III | IV | V | VI |
|---|---|---|---|---|---|---|
| % Recovery | 20.59+2.2[a] | 40.37+2.3[b] | 27.04+2.5[c] | 12.20+1.1[d] | 1.48+0.8[e] | 1.09+0.3[f] |
| % DPPH* scavenging activity/mg of powder | 32.77±0.5[a] | 17.27±0.3[b] | 44.61±0.8[c] | 29.91±1.1[d] | 149.86±0.9[e] | 13.1±0.7[f] |
| Gallic acid (mg/g) | ND | ND | 17.15±1.1 | ND | ND | ND |
| Catechin (mg/g) | ND | ND | 424.92±1.4 | ND | ND | ND |
| Syringic acid (mg/g) | ND | ND | ND | 18.95+1.0 | 4.34+0.1 | ND |
| Caffeic acid (mg/g) | ND | ND | ND | 10.19+ 0.8 | | ND |

**Note:** Mean + standard deviation; Observation with different superscript in the same row differs significantly at 5% probability level.

**Figure 4: FTIR Spectra of Different Fractions of Amla Pomace Powder. REA: Aqueous Extract, REA: Ethyl Acetate Extract, RB: Butanolic Extract, I: Fraction I, II: Fraction II, III: Fraction III, and V: Fraction V**

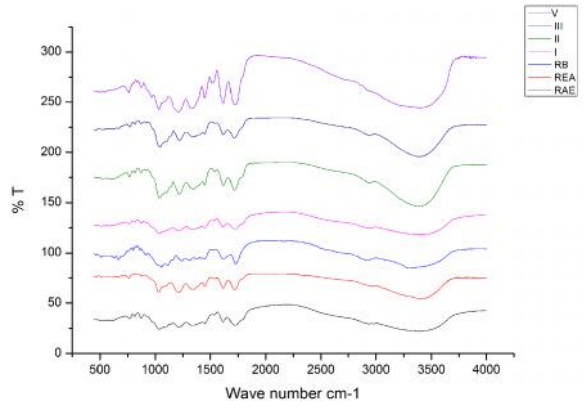



**Figure 5: HPLC Chromatogram of (a) E-1 (Hydroxytyrosol), (b) E-2 (Gallic Acid), and (c) E-3 (Catechin) at 280 nm**

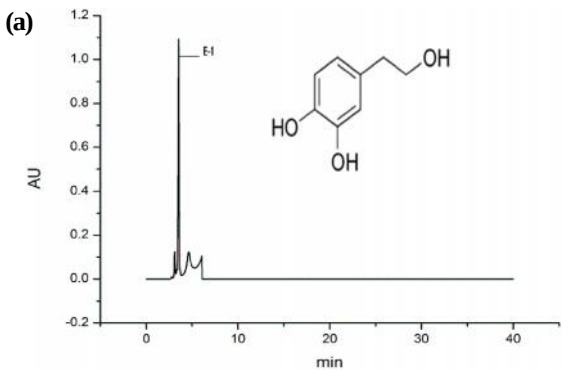
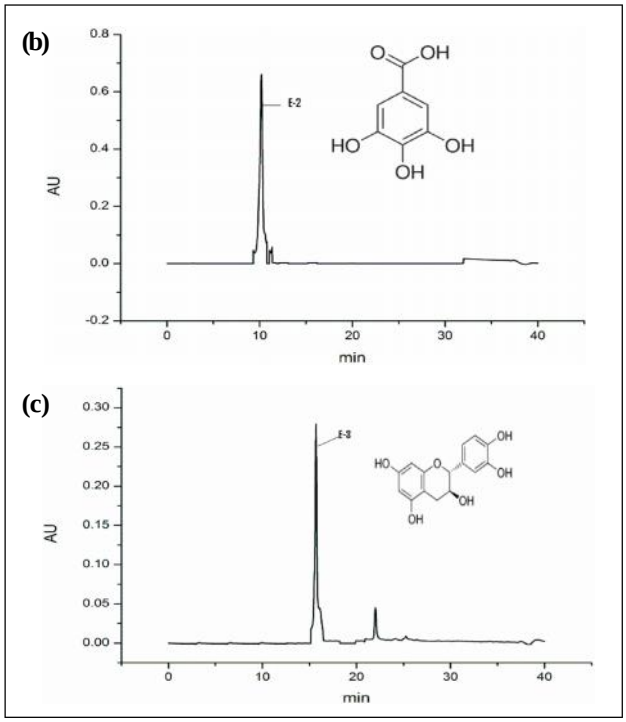



Figure 5 (Cont.)




isolated compound were obtained at 73, 267, 193 and 179. The present finding was also in accordance with Proestos *et al.* (2006).

## DPPH* Scavenging Activity

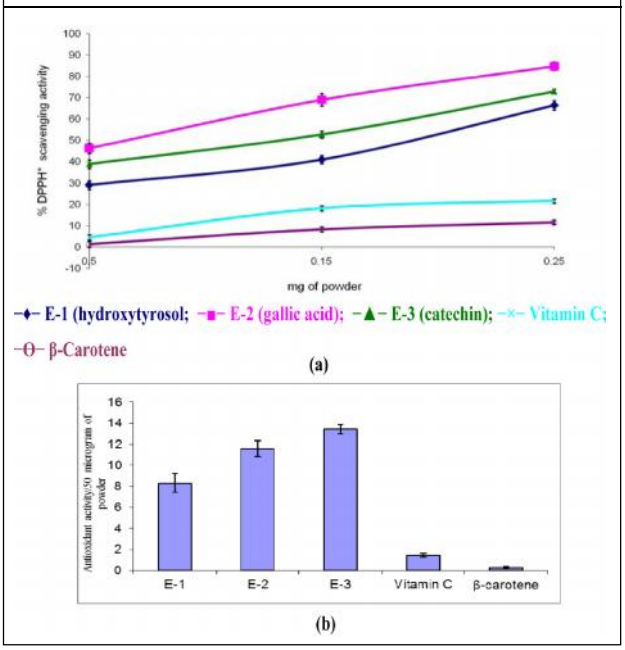
Ethyl acetate fraction showed significantly less total phenolic content than the butanolic fraction of the Amla pomace powder. Probably during evaporation of butanol solvent, some other phenolics may have been synthesized from the secondary metabolites which may increase the

total phenolic content of the butanolic fraction. However inspite of having good amount of total phenolic content, the DPPH* scavenging activity was significantly less than the ethyl acetate fraction. Our present findings contradicted Piljac-Zegarac *et al.* (2007) and Seruga *et al.* (2011) for their observation that the DPPH* scavenging activity is directly proportional to the total phenolic content. The DPPH* scavenging activity and antioxidant activity not only depends on the total phenolic content but also depends on the position and availability of the free hydroxyl groups to show antioxidant activity or to scavenge the free radicals. Exposure of high temperature to evaporate the solvents from butanolic extracts adversely affects the availability of hydroxyl groups that reduces the functional properties of the phenolic compound present and hence was not found suitable for the extraction of bioactive components. Fraction V (Table 2) showed the highest DPPH* scavenging activity among all fractions tested but due to comparatively poor recovery of phenolics could not be recommended for the extraction of bioactive components. It can also be concluded from Figure 4 that FTIR spectra of fraction V had comparatively higher intensity of functional peaks as compared to others. Luo and Foo (2001) explained that such hydroxy-phenolic compounds can give hydrogen atoms to DPPH* and can scavenge it. Fraction III with significant recovery of the phenolics and compatible DPPH* free radical scavenging activity may be suggested for the further extraction and purification of the components.

The DPPH* scavenging activities of compound E1-E3, and control are shown in Figure 6a. It could be observed that all three compounds have strong DPPH* scavenging activity in a dose dependent manner. The DPPH* scavenging activity was in declining order; gallic acid (E-2) > catechin (E-3) > hydroxytyrosol (E-1)> vitamin C > â carotene. As shown in Figure 6, the DPPH* scavenging activities of the isolated compounds were significantly higher than those of vitamin C and â-carotene.

Figure 6b presents the $ABTS^{+}$ scavenging activities of the isolated compounds and standards of vitamin C and β-carotene. Catechin, gallic acid and hydroxytyrosol exhibited potent free radical scavenging activities and these findings were consistent with Giedrius-Miliauskas *et al.* (2004) and Luo *et al.* (2009). Moreover, gallic acid, catechin and hydroxytyrosol exhibited higher free radical scavenging activities than the vitamin C and β-carotene standards. The $ABTS^{+}$ scavenging activity in declining order was as follows: catechin > gallic acid > hydroxytyrosol > vitamin C > β carotene. This result confirmed the idea that a high free radical scavenging activity can be credited to the availability of their free hydroxyl groups, and the phenolic hydroxyl structural group in benzene ring contributes much to the antioxidant activity (Shaidi *et al.*, 1992; and Luo *et al.*, 2009).

**Figure 6: (a) DPPH* Scavenging Activity of Isolated Components (E-1 to E-3 ), Standard Vitamin C and -carotene, and (b) ABTS+ Scavenging Activity of Isolated Compounds (E-1 to E-3), Standard Vitamin C and Standard -carotene**




## CONCLUSION

Major polyphenols contained in ethyl acetate fractions of pomace powder were gallic acid, catechin, caffeic acid and syringic acid. Ethyl acetate fraction extracted by methanol:water (80:20) showed the maximum DPPH* scavenging activity but due to very poor recovery it was not found suitable for the further purification. Catechin was the dominating acid present in fraction III. Isolated catechin, gallic acid and hydroxytyrosol exhibited stronger DPPH* free radical scavenging activity as compared to vitamin C and β-carotene. Present study revealed that most of the major fractions of the crucial bioactive components of Amla are present in the pomace powder which can be exploited after extraction of juice from the fruit.

## REFERENCES

- Cai Y, Luo Q, Sun M and Corke H (2004), "Antioxidant Activity and Phenolic Compounds of 112 Traditional

Chinese Medicinal Plants Associated with Anticancer", *Life Sci*., Vol. 74, pp. 2157-2184.

- Das D, Bandyopadhyay D, Bhattacharjee M and Banerjee R K (1997), "Hydroxyl Radical is the Major Causative Factor in Stress Induced Gastric Ulceration", *Free Radical Biol. Med*., Vol. 22, pp. 749-760.
- E1-Deousky S K, Ryu S Y and Kim Y K (2008), "A New Cytotoxic Acylated Apigenin Glucoside from *Phyllanthus emblica* L.", *Nat Prod Res.*, Vol. 22, pp. 91-95.
- Giedrius-Miliauskas T A, Van Beek-Petras R, Venskutonis-Jozef P H and Linssen-Pieter W (2004), "Antioxidative Activity of Geranium Macrorrhizum", *Euro Food Res. Technol*., Vol. 218, pp. 253-261.
- Husain S R, Cillard J and Cillard P (1987), "Hydroxyl Radical Scavenging Activity of Flavonoids", *Phytochem*, Vol. 26, pp. 2489-2491.
- Ikawa M, Schaper T D, Dollard C A and Sasner J J (2003), "Utilization of Foline Ciocalteu Phenol Reagent for the Detection of Certain Nitrogen Compounds", *J. Agri Food Chem*., Vol. 51, No. 7, pp. 811-1815.
- Kahkonen M P, Hopia A I, Vuorela H J, Rauha J P, Pihlaja K, Kujala T S and Heinonen M (1999), "Antioxidant Activity of Plant Extracts Containing Phenolic Compounds", *J. Agri Food Chem.*, Vol. 47**,** pp. 3954-3962.
- Kusirisin W, Srichairatanakool S, Lerttrakarnnon P, Lailerd N, Suttajit M and Jaikang C (2009), "Antioxidative Activity Polyphenolic Content and Antiglycation Effect of Some Thai Medicinal Plants Traditionally Used in Diabetic Patients", *Medicinal Chem*., Vol. 5, No. 2, pp. 139-147.
- Liu X, Zhao M, Wu K, Chai K, Yu H, Tao Z and Wang J (2012), "Immunomodulatory and Anticancer Activities of Phenolics from Emblica Fruit (*Phyllanthus emblica* L.)", *Food Chem*., Vol. 131, pp. 685-690.
- Lu Y and Foo L Y (2001), "Antioxidant Activity of Polyphones from Sage (*Salvia officinalis*)", *Food Chem*. Vol. 75, pp. 197-202.
- Luo W, Zhao M, Yang B, Ren J, Shen G and Rao G (2011), "Antioxidant and Antiproliferative Capacities of Phenolics Purified from *Phyllanthus emblica* L. Fruit", *Food Chem*., Vol. 126, pp. 277-282.
- Luo W, Zhao M, Yang B, Shen G and Rao G (2009), "Identification of Bioactive Compounds in *Phyllanthus emblica* L. Fruit & their Free Radical Scavenging Activity", *Food Chem*., Vol. 114, pp. 499-504.
- Mayachiew P and Devahastin S (2010), "Effects of Drying Methods and Conditions on Release Characteristics of Edible Chitosan Films Enriched with Indian Gooseberry Extract", *Food Chem*., Vol. 118, pp. 594-601.
- Muramatsu H, Kogawa K, Tanaka M, Okumura K, Koike K and Kuga T (1995), "Superoxide Dismutase in SAS Human Tongue Carcinoma Cell Line is a Factor Defining Invasiveness and Cell Motility", *Cancer Res*., Vol. 55, pp. 6210-6214.
- Nirmaladevi R, Padma P R and Kvitha D (2010), "Analyses of the Methanolic Extracts of the Leaves of *Rhinacanthus nasutus*", *J. Med. Plants Res*., Vol. 4, pp. 1554-1560.
- Oliver C N, Ahn B, Moerman E J, Goldstein S and Stadtmaan E R (1987), "Age Related Changes in Oxidized Protein", *J. Biol. Chem*., Vol. 262, pp. 5488-5491.
- Olivier M, Vandeputte M, Sanda M K, Diallo R, Modher B, EI Jaziri A M and Baucher E I (2010), "Identification of Catechin as One of the Flavonoids from *Combretum albiflorum* Bark Extract that Reduces the Production of Quorum-Sensing Controlled Virulence Factors in *Pseudomonas aeruginosa* PA 01", *Applied Environ. Microbiol*., Vol. 76, No. 1, p. 243.
- Piljac-Zegarac J, Martinez S, Valek L, Stipc¡evic T, Kovac¡evic-Ganic K (2007), "Correlation Between the Phenolic Content and DPPH Radical Scavenging Activity of Selected Croatian Wines", *Acta Aliment*., Vol. 36, pp. 185-193.
- Proestos C, Sereli D and Komaitis M (2006), "Determination of Phenolic Compounds in Aromatic Plants by RP-HPLC and GC-MS", *Food Chem*., Vol. 95, pp. 44-52.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C (1999), "Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay", *Free Rad Biol Med*., Vol. 26, pp. 1231-1237.
- Robak J and Dryglewski R J (1988), "Flavonoids are Scavengers of Superoxide Anion", *Biochem Pharmacol*., Vol. 37, pp. 83-88.

- Roby M H H, Sarhan M A, Selim K H A and Khalel K I (2013), "Evaluation of Antioxidant Activity, Total Phenols and Phenolic Compounds in Thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and Marjoram (*Origanum majorana* L.) Extracts", *Indus Crops Prod*., Vol. 43, pp. 827-831.
- Seruga M, Navak I and Jakobek L (2011), "Determination of Polyphenolic Content and Antioxidant Activity of Some Red Wines by Differential Pulse Voltametry; HPLC and Spectrophotometric Method", *Food Chem*., Vol. 124, pp. 1208-1216.
- Shaidi F, Janitha P K and Wanasundara P D (1992), "Free Radical Scavenging Activity of Phenolics are Mainly Due to the Presence of Free Hydroxyl Groups Phenolic Antioxidants", *Critical Reviews Food Sci. Nutri.*, Vol. 32, pp. 67-103.
- Smith M A, Perry G, Sayre L M, Anderson V E, Beal M F and Kowall N (1996), "Oxidative Damage in Alzheimer's", *Nature*, Vol. 382, pp. 120-121.
- Steinberg D, Parthasarathy S, Carew T E, Khoo J C and Witztum J L (1998), "Beyond Cholesterol, Modification of Lowdensity Lipoprotein that Increase its Atherogenicity", *New Engl J. Med*., Vol. 320, pp. 915-924.
- Torel J, Cillard J and Cillard P (1986), "Antioxidant Activity of Flavonoids and Reactivity with Peroxy Radicals", *Phytochem*., Vol. 25, pp. 383-385.
- Vinson J A, Su X, Zubik L and Bose P (2002), "Phenol Antioxidant Quantity and Quality in Foods: Fruits", *J. Agri. Food Chem*., Vol. 49, No. 11, pp. 5315-5321.
- Wang H, Jenner A M, Lee C Y, Shui G, Tang S Y, Whiteman M, Wenk M R and Halliwell B (2007), "The Identification of Antioxidants in Dark Soy Sauce", *Free Rad Res*., Vol. 41, pp. 479-488.
- Zhang L Z, Zhao W H, Gua Y J, Tu G Z, Lin S and Xin L G (2003), "Studies on Chemical Constituents in Fruits of Tibetan Medicine *Phyllanthus emblica*", *Zhongguo Zhong Yao Za Zhi*., Vol. 28, pp. 940-943.


IJFANS
International Journal of Food
And Nutritional Sciences