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LCMS STUDY OF THE INDIGENOUS FRUITS AND VEGETABLES OF INDIAN HIMALAYAN REGION

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ABSTRACT

Phenolics of *Prunus avium*, *Cydonia oblonga* & *Malva neglecta* and carotenoids of *Taraxacum officinale* of the selected indigenous fruits & vegetables of Indian Himalayan region (IHR) have been characterized and analysed for the first time. Phenolics & carotenoids were determined by LCMS/MS using a reverse phase C18 column. In *Prunus avium,* the phenolics like cyanidins, rutin, epicatechin & catechin were found. In *Cydonia oblonga*, the caffeoyl quinic acids were abundant. A total of 24 carotenoids were identified in *Taraxacum officinale*. The most important identified were (all-Z)-β-cryptoxanthin, antheraxanthin, (all-E & Z)-lutein, (all-E)-zeaxanthin and (all-Z)-violaxanthin. Hydrocarbon carotenoids found were (all-E)-β-carotene, (9Z)-β-carotene & (13Z)-β-carotene. In *Malva neglecta*, the major phenolics were quinic acid, chlorogenic acid, rutin, & gallic acid. This work will contribute to the development of scientific research about the bioaccessibility and bioavailability of phenolics & carotenoids for better understanding of the phenolics and carotenoids impact on human health.

1.Introduction

The importance of diversity of fruits and vegetables in human diet to maintain good health is well established. Fruit and vegetable consumption is recommended in numerous food-based dietary guidelines and forms a key recommendation in many international statements related to healthy diets. There is enormous potential to better incorporate the wealth of diverse fruit and vegetable species into food systems. Indigenous fruits and vegetables are defined as locally-produced, socially and culturally accepted as local foods and eaten by previous generations or introduced for a very long time. They provide a variety of products like food, medicines, raw materials and are also an important source of renewable energy.

Chronic diseases like coronary heart diseases, cancers, immune dysfunction and diabetes mellitus are found less in people with increased fruit & vegetable consumption (Pandey et al., 2009). Fruits & vegetables being rich in health-promoting nutrients like vitamins, minerals, phenolics and flavonoids act as a primary food source. In addition to commercial fruits, edible wild fruits have drawn attention due to their exceptional antioxidant properties and increased polyphenol levels (Murillo et al., 2012; Siqueira et al., 2013). The Northern Himalayan tribes and rural residents rely heavily on wild edible fruits for their nutrition (Samant et al., 2001). For thousands of years, they have been used both as food and medicine (Gaur et al., 1999). Limited studies on wild edible fruits

& vegetables of Kashmir valley of North Himalayas have shown presence of abundant polyphenols and associated antioxidant, anti-microbial, anti-inflammatory and anti-proliferative activities (Badhani et al., 2015; Singh et al., 2015). *Prunus avium* & *Cydonia oblonga* as fruits and *Taraxacum officinale & Malva neglecta as vegetables* are the underutilized edible plants of Indian Himalayan region (IHR) selected for this study. In J &K, these plant species are abundantly found (Maikhuri et al., 2004).

One of the most liked temperate fruits is the sweet cherry (*Prunus avium*), which is well regarded by consumers and extensively researched by scientists for its flavour, colour, sweetness, nutritional and bioactive qualities. Sweet cherry fruits contain a variety of phenolics, including flavonoids (anthocyanins, flavan-3-ols, and flavonols) and phenolic acids (hydroxycinnamic derivatives), possessing antioxidant activity ((Ballistreri et al., 2013; Pacifico et al., 2014).

Quince fruit (*Cydonia oblonga*) is too acidic, astringent and tough to be consumed fresh. This fruit is readily accessible and a reasonably priced dietary source of phytoconstituents with positive health effects (Mir et al., 2016). Recent pharmacological studies have revealed that quince fruit has antioxidant, antibacterial, antiviral, anti-inflammatory, anti-ulcer, anticancer, and antihaemolytic properties, suggesting potential interest for pharmaceutical and nutraceutical uses. (Carvalho et al., 2010). Procyanidins are the other main constituents in quince fruits, and such compounds are known for their antioxidant, cardiac-vascular & cancer-related effects (Crozier et al., 2009). The large amount of phenolics and long use in traditional medicine of quince fruit prompted us to consider this natural product as a valuable source of phytoconstituents to be exploited in nutraceutical products (Patel et al., 2011).

Taraxacum officinale commonly known as dandelion, is a well-known herb that is consumed all over the world. Due to its wealth of nutrients, *Taraxacum* can also be utilised as a vegetable in addition to being a medicinal plant (Bajaj 1994). *Taraxacum* consumption has been shown to be protective against liver and lung damage in rats (Domitrovic et al., 2010; Liu et al., 2010). Numerous functional elements, including taraxacerin, triterpenoid, chlorogenic acid, saponin, and carotenoid, are responsible for this significant biological activity (Shi et al., 2008). Researchers have focused on *Taraxacum* species because of their antioxidant potential in addition to its analgesic, anti-inflammatory, anticarcinogenic, anti-allergic, anti-hyperglycemic and anti-inflammatory properties (Bajaj 1994). Furthermore, locals in the Northern Himalayas have consumed fresh *T. officinale* leaves as a vegetable diet. Additionally, because of its purported medical qualities, extracts are employed as flavouring agents in a variety of food products, such as alcoholic beverages, soft drinks, frozen dairy desserts, candies, baked goods and gelatins.

Malva neglecta is one of the most used herbal medicines. It is an edible plant wherein its boiled leaves are taken as wholesome vegetable. Leaves and roots of *M. neglecta* are used in traditional medicine for wound healing in several countries of the world (Ozudogru et al., 2011). The leaf possesses the ability to treat diabetes, cough, gynaecological diseases, and stomach aches. The herb is used to cure dermatitis, fractured bones, burns, and throat infections (Bushra et al., 2012).

Phenolic compounds constitute a large and heterogeneous class of compounds with a very wide distribution in taxa of higher plants. Despite this almost ubiquity, experimental evidence has demonstrated that each plant species is characterized by the presence of a limited number of compounds. Within each species, the nature of these compounds can vary from organ to organ but is constant enough toward several other factors. These facts have been used in recent years, in the characterization of several food products of plant origin by their phenolic profile. Factors contributing to the variability in phenolic distribution include cultivar and genetics, geographical origin, maturity, climate, position on tree and agricultural practices (Spanos et al., 1992).

The large amount of phenolics & carotenoids and long use in traditional medicine

of the selected minor fruits & vegetables prompted us to consider natural products as a valuable source of phytoconstituents to be exploited in nutraceutical products. The aim of the present work was to explore and characterize the polyphenolic & carotenoid composition of the selected indigenous fruits and vegetables by liquid chromatography–mass spectroscopy (LC-MS) analysis.

2. Materials & Methods

2.1. Chemicals and Reagents

MS grade formic acid, methanol, water & acetonitrile were purchased from Fisher Scientific (Mumbai, India). Double- deionised water from a Milli-Q-system from Millipore (Elix Technology, Bangalore India) was used. The standard compounds Quinic acid, Malic acid, tr-Aconitic acid, Gallic acid, Chlorogenic acid, Protocatechuic acid, Tannic acid, tr-Caffeic acid, Vanillin, P- Coumaric acid, Rosmarinic acid, Rutin, Hesperidin, Hyperoside, 4-OH-Benzoic acid, Salicylic acid, Myricetin, Fisetin, Coumarin, Quercetin, Naringenin, Hesperidin, Luteolin, Kaempferol, Apigenin, Rhamnetin, Chyrsin, 3-O-Caffeoyl quinic acid, 4-O-Caffeoyl quinic acid, 5-O-Caffeoyl quinic acid, 3,5-di Caffeoyl quinic acid, Quercetin 3- galactoside, Kaempferol glycoside, Kaempferol 3-glycoside, Kaempferol 3-Rutinoside, Neochlorogenic acid, P-Coumaryl quinic acid, Cyanidin 3- glucoside, Cyanidin 3- rutinoside, Peonidin 3- glucoside, Pelargonidin 3- rutinoside, Peonidin 3-rutinoside, Catechin, Epicatechin, 9- Cis violaxanthin, Neochrome, All- Trans-neoxanthin, All-Trans-violaxanthin, 9-Cis-neoxanthin, Luteoxanthin, Cis-violaxanthin, Antheraxanthin, 9-Cis-violaxanthin, 13- Cis-lutein, All Trans lutein, All Trans zeaxanthin & 9 Cis- lutein, were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. Plant collection and Extraction

The selected minor fruits and vegetables were collected from fields of the Pulwama district of J&K, India. Plants aerial part (leaves & fruits) were thoroughly washed to remove dust and superfluous material with distilled water and shade dried for two weeks. Then it was crushed into powder form by a mechanical grinder. The coarse powder (1 kg) was subjected to maceration with 1500 mL of 70% methanol for seven days in air tight container with occasional shake at room temperature. The macerate was passed through muslin cloth and then filtered through whatman filter paper no. 1. The filtrate was concentrated via rotary evaporator (Heidolph Laborota 4000, Schwabach, Germany) under reduced pressure at 35°C, which resulted in the formation 120 gm (12% yield) semisolid crude extract (Ullah et al., 2016; Zohra et al., 2019). The extract (100 mg) was re-dissolved in methanol (5 mL) and vortexed for 1 min. The final extracts were filtered with regenerated cellulose filters 0.2 µm, (Millipore, Bedford, MA, USA) to 2 mL HPLC vials for analysis.

2.3. Methodology

All the retention times and MS data were collected using the C_{18} Accucore aQ column (100×2.1mm, Particle size 2.6µ, Part No. 17326-102130). For MS measurements, a positive/negative switching ion mode was used to obtain better tandem mass spectra and high resolution mass spectra. For all the compounds the high resolution mass data was in good agreement with the theoretical molecular formulas, all displaying a mass error of below 5 ppm thus confirming their elemental composition. In general, peak identities were consistent both within and between analyses. Fragment ions with intensities between 10% of the base peak were reported only when they were needed for comparison. The phenolics were positively identified by their typical UV-absorptions at 254, 280 and 320 nm.

2.4. LC Parameters

Qualitative analysis was conducted on a Quantum triple stage quadrupole (TSQ) mass spectrometer, equipped with a quaternary solvent delivery system, a column oven, a photo-diode array detector and an autosampler. An aliquot (10 µl) of each methanolic extract was chromatographed on a 100 × 2.1 mm, particle size 2.6 µm Accucore aQ (Part No. 17326-

102130) C_{18} column which was heated to 30 °C. Analytes were separated using 0.1% formic acid + 5 mM ammonium formate in purified water (Mobile Phase A) and 0.1% formic acid + 5 mM ammonium formate in Methanol (Mobile Phase B) under a flow rate of 0.3 ml/min. The gradient employed was 0% B for 2 min, followed by an increase to 100% B over 18 min, and then hold for 1 min. Ions for mass spectrometry were generated using an electrospray source in either the positive or negative mode (depending on analyte). MS experiments in the full scan (parent and product-specific) and the selected reaction monitoring (SRM) mode were conducted.

2.5. MS Parameters

Mass Spectrometry (MS) analysis was performed on a Thermo Scientific TSQ Endura (TQH-E-1-0565) mass spectrometer with ESI source (Thermo Fisher Scientific, United States). The ionization interface was operated in both positive-ion (PI) electrospray mode for carotenoids and negative ion (NI) mode for other polyphenol compounds. The conditions were the same both in PI and NI electrospray modes. Source parameters were as follows: Sheath gas 30; Ion transfer tube temperature 200°C; vaporizer temperature 300 °C; auxiliary gas 10; sweep gas 1. Scan source parameters: positive ion spray voltage, 3500 V; negative spray voltage 2800 V. The following conditions were used for MS: scan range, 100-1000 (m/z); MS scan rate, 1.0 second; MS/MS scan rate, 4.0 seconds; and collision energy, 30 V. Dual source technology was applied for mass accuracy. ESI low concentration tuning mix was used to adjust the mass calibration of the instrument during analysis. Data was processed by Thermo LC Quan software.

3. Results and discussions

Table 1. MS^n fragmentation of *Prunus avium* phenolics

Peak No.	Compound	Molecular ion m/z [M-H]	MS^n m/z (C.E)
01	Neochlorogenic acid	354	191
02	p- Coumaryl quinic acid	337	163, 191
03	Chlorogenic acid	354	191(100), 179(60), 173(5), 135(50)
04	Cyanidin-3-O-glucoside	449	287(100)
05	Cyanidin-3-O-rutinoside	595	449(10), 287(100)
06	Peonidin-3-O-glucoside	464	463(8), 301(100)
08	Peonidin-3-O-rutinoside	609	463(8), 301(100)
09	Catechin	289	MS^2 → 245 (100), 205 (23), 179 (23); MS^3 → 203 (100), 227 (17), 189 (12), 161 (18)
10	Epicatechin	289	MS^2 → 245 (100), 205 (35), 179 (15); MS^3 → 203 (100), 227 (18), 189 (17), 161 (28)
11	Rutin	609	301

C.E= Collision Energy for ms^n Transition

Table 2. MS^n fragmentation of *Cydonia oblonga* phenolics

Peak No.	Compound	Molecular ion m/z [M-H]	MS^n m/z (C.E)
01	3-O-Caffeoylquinic acid	354	MS^2 → 191 (100); MS^3 → 127 (100), 173 (34), 85 (94) 109 (27); MS^4 → 109 (100)
02	4-O-Caffeoylquinic acid	354	MS^2 → 191 (100); MS^3 → 173 (76), 127 (61), 85 (87), 93 (49); MS^4 → 111 (100) 3)
03	5-O-Caffeoylquinic acid	354	MS^2 → 179 (100), 135 (19), 161 (2); MS^3 → 135 (100)
04	3,5 Dicaffeoyl quinic acid	516	MS^2 → 245 (100), 205 (23), 179 (23); MS^3 → 203 (100), 227 (17), 189 (12), 161 (18)
06	Kaempferol 7-O-glucoside	447	MS^2 → 285 (55), 284 (100), 255 (27); MS^3 → 255(100), 267(20); MS^4 → 255 (100), 163 (30), 227 (34)

C.E= Collision Energy for ms^n Transition

Table 3. MS^n Fragmentation of Carotenoids of *Taraxacum officinale*

Peak No.	Compound Name	Parent ion m/z $[M+H]^+$	Fragments MS^n m/z
01	9-or 9'-cis-Violaxanthin	601.5	583 $[M+H-18]^+$, 565 $[M+H-36]^+$, 509 $[M+H-92]^+$, 491$[M+H-92-18]^+$
02	Neochrome	601.5	583 $[M+H-18]^+$, 399,421,477.
03	Trans Neoxanthin	601.4	583.4$[M+H-18]^+$, 565 $[M+H-36]^+$, 547$[M+H-54]^+$, 521$[M+H-80]^+$
04	Trans Violaxanthin	601	583$[M+H-18]^+$, 565 $[M+H-36]^+$, 521$[M+H-80]^+$
05	9-or 9'-cis-Neoxanthin	601	583 $[M+H-18]^+$, 565 $[M+H-36]^+$, 547$[M+H-54]^+$, 521$[M+H-80]^+$
06	Luteoxanthin	601	583 $[M+H-18]^+$
07	Cis Violaxanthin	601	583 $[M+H-18]^+$, 565 $[M+H-36]^+$
08	Antheraxanthin	585	567 $[M+H-18]^+$, 549 $[M+H-36]^+$, 505$[M+H-80]^+$
11	13, 13 – cis- Lutein	569	551 $[M+H-18]^+$, 533 $[M+H-36]^+$
17	β- Cryptoxanthin	553	535 $[M+H-18]^+$, 461$[M+H-92]^+$
20	15, 15' cis- β-Carotene	537	457 $[M+H-80]^+$, 413$[M+H-124]^+$, 123$[M+H-414]^+$, 177$[M+H-360]^+$, 137 $[M+H-400]^+$
21	9, 9' cis- β-Carotene	537	457 $[M+H-80]^+$, 445$[M+H-92]^+$

23	13-or 13'-cis-β-Carotene	537	457 [M+H−80]$^+$, 445[M+H−92]$^+$, 400 [M+H−137]$^+$, 269[M+H−268]$^+$, 177[M+H−360]$^+$, 137 [M+H−400]$^+$
24	β-Carotene	537	481 [M+H−56]$^+$, 445[M+H−92]$^+$

Table 4. MS^n Fragmentation of phenolic compounds of methanol extracts of *Malva neglecta*

Peak. No.	Compound	Parent ion (m/z)	MS^2 (C.E)
01	Quinic acid	190.95	173, 127, 109, 85 (22),93 (22)
02	Malic acid	133.05	115 (14),71 (17)
04	Gallic acid	169.05	125 (14),79 (25)
05	Chlorogenic acid	353	191 (17
07	Tannic acid	182.95	124 (22),78 (34)
09	Vanillin	151.05	136 (17),92 (21)
10	p-Coumaric acid	162.95	119 (15),93 (31)
12	Rutin	609.1	300 (37), 271 (51), 301 (38)
13	Hesperidin	611.1	303,465
15	4-OH Benzoic acid	136.95	93,65
16	Salicylic acid	136.95	93,65,75
19	Coumarin	146.95	103,91,77
20	Quercetin	300.9	179,151,121
23	Luteolin	284.95	175,151,133
24	Kaempferol	284.95	217,133,151

C.E= Collision Energy for ms^n Transition

The qualitative determination of secondary metabolites of the selected indigenous fruits (*Prunus avium* & *Cydonia oblonga*) and vegetables (*Taraxacum officinale* & *Malva neglecta*) was performed by LCMS/MS and allowed identification of phytochemicals like organic acids, hydroxycinnamic and caffeic derivatives, catechin, procyanidins, and flavonols**.** Table 1, 2, 3 & 4 shows the identification data containing mass spectra of parent compound and fragments of *Prunus avium, Cydonia oblonga*, *Taraxacum officinale* **&** *Malva neglecta*.

3.1. *Prunus avium*

Figures 1 & 2 shows the HPLC chromatogram of standards mixture and methanol extract of *Prunus avium*. ESI–MS^n mode revealed the presence of peak 1 with molecular ion peak at 354 m/z.

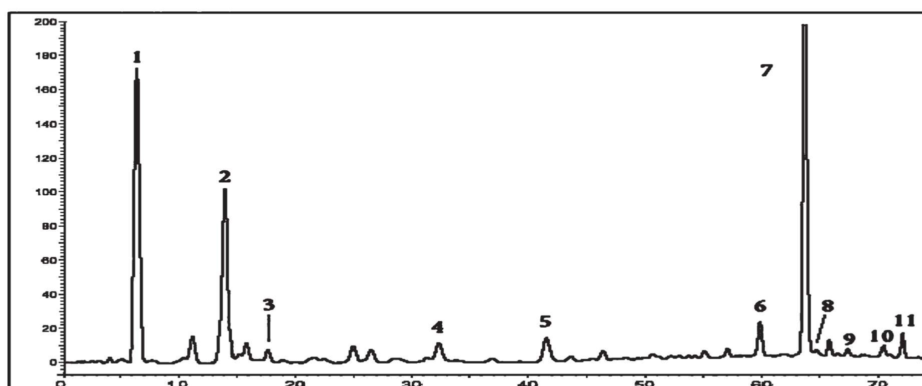

Figure. 1. HPLC profile of standards mixture of *Prunus avium*

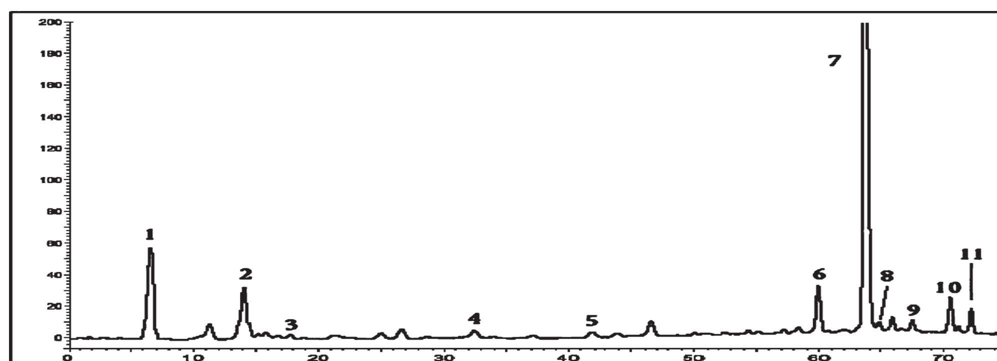

Figure 2. HPLC chromatograms of *Prunus avium* extract

The major fragments were at m/z 191. Based on molecular mass and fragmentation pattern, peak 1 was tentatively identified as Neochlorogenic acid. Peak 2 with molecular ion peak at 337 and m/z of its fragments as 163 & 191 was identified as p- Coumaryl quinic acid. Peak 3 having m/z 353 and fragment m/z as 191, 179, 173 & 135 was identified as Chlorogenic acid. Peak 4 with m/z 485 and m/z of its fragment as 287 identified as Cyanidin -3-glucoside. Peak 5 with m/z as 595 and fragments m/z as 449 & 287 identified as Cyanidin 3-rutinoside. Peak 6 with m/z as 463 and its fragment m/z 301 identified as Peonidin 3-glucoside. Peak 8 with m/z 609 with fragments m/z 463 & 301identified as Peonidin 3-rutinoside. Peak 9 & 10 with m/z 289 and its fragments m/z 245, 205, 179,203, 227, 189 & 161 was identified as Catechin & Epicatechin. Peak 11 with m/z 609 and its fragments m/z 301 identified as Rutin.

3.2. *Cydonia oblonga*

Figures 3 & 4 shows the HPLC chromatogram of standards mixture and methanol extract of *Cydonia oblonga*. ESI–MS^n mode revealed the presence of peaks 1, 2 &3 as isomers with molecular ion peak at 354 m/z. The major fragments were at m/z 191. Based on molecular mass and fragmentation pattern, peaks 1, 2 & 3 were tentatively identified as 3-O- caffeoyl quinic acid, 4-O-caffeoyl quinic acid & 5-O-caffeoyl quinic acid. Peak 4 with molecular ion peak at 516 and m/z of its fragments is 163 & 191 was identified as 3, 5 dicaffeoyl quinic acid. Peak 6 with m/z 447 and its fragmentation with m/z 285, 255, 267 identified as Kaempferol 7-O-glucoside.

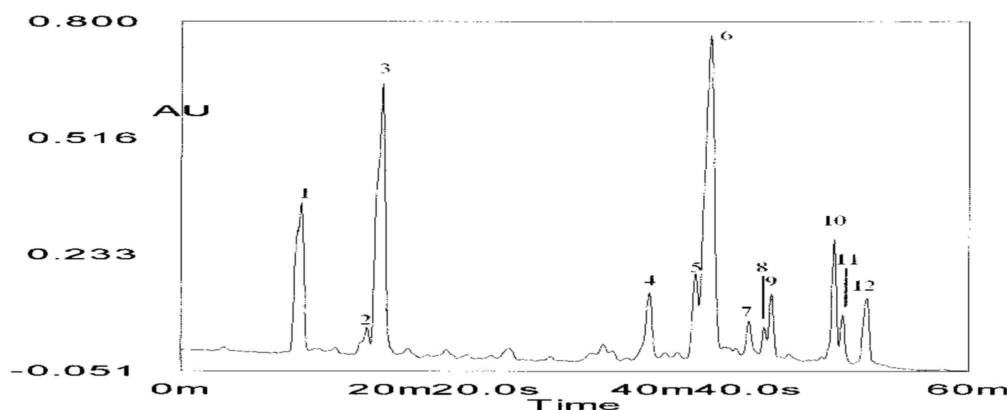

Figure 3. HPLC profile of a *Cydonia oblonga* standards mixture

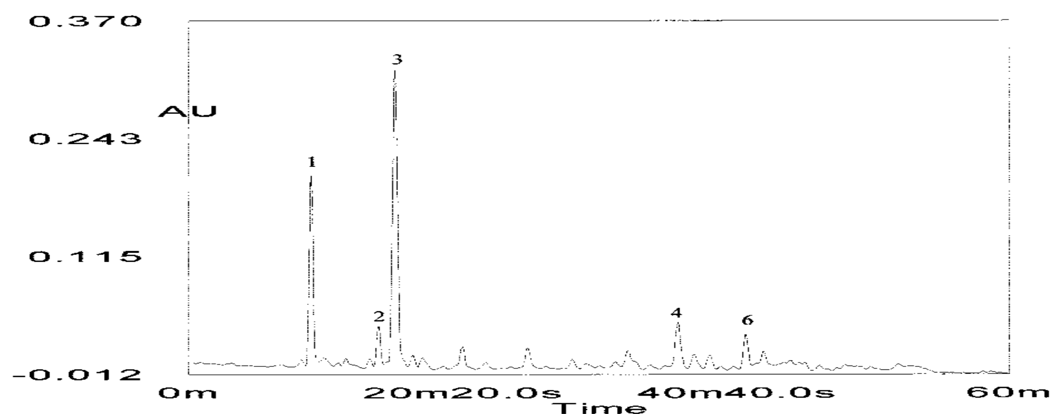

Figure 4. HPLC profile of *Cydonia oblonga* extract

3.3. Taraxacum officinale

Figures 5 & 6 shows the HPLC chromatogram of standards mixture and methanol extract of *Taraxacum officinale*. Peak 1 was identified as 9- or 9' –Cisviolaxanthin having $[M + H]^+$ at m/z of 601.5. The loss of one molecule of water leads to $[M + H-18]^+$ at m/z of 583 and loss of two water molecules to $[M + H -18-18]^+$ at m/z of 565. Loss of toluene molecule leads to $[M + H -92]^+$ at m/z of 509 and loss of toluene & water molecule to $[M + H -92-18]^+$ at m/z of 491. Peak 2 was identified as Neochrome due to hypsochromic shift of about 20 nm and m/z of protonated $[M+H]^+$ 601.5 with that reported by de Faria et al (2009). Both peaks 3 & 4 were identified as all Trans forms of neoxanthin and violaxanthin respectively based on epoxide test, absorption spectra and mass spectra characteristics. Peak 5 was identified as 9, 9' Cis- neoxanthin as a hypsochromic shift of about 6 nm and a weak absorption band at 326 nm occurred. Peak 6 with $[M + H]^+$ m/z 601 was identified as Luteoxanthin. This 5, 8- epoxy xanthophyll was characterized by the major product ion at m/z 583 $[M + H-18]^+$ and at m/z 221, resulting from the cleavage of C_{10}-C_{11} bond in the polyene chain from the epoxy end group. The diagnostic ion at m/z 221 indicated the presence of an epoxy substituent in a b-ring with a hydroxyl group (Britton et al., 2004). The ion at m/z 221 corresponds to the oxo-ring fused to the 3-hydroxy-b-ring. Compound 7 exhibited $[M +H]^+$ at m/z 601 and MS^2 fragment at m/z 583 and was identified as Cis- violaxanthin. The mass spectrum displayed fragments at 583 $[M + H-18]^+$, 565 $[M + H-18-18]^+$ which correspond to the loss of one water molecule and two water molecules respectively. Peak 8 with protonated $[M + H]^+$ m/z at 601 and fragments at m/z 567 $[M+H -18]^+$ resulting from the loss of a water molecule and also a fragment at m/z 493 $[M + H-92]^+$which correspond to the loss of toluene.

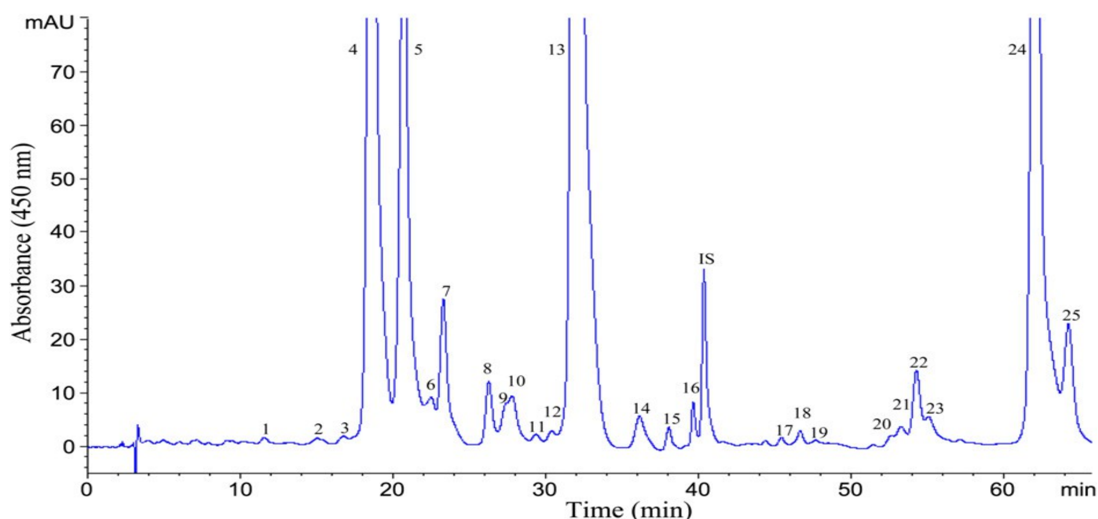

Figure 5. HPLC chromatogram of standards mixture of *T. officinale*

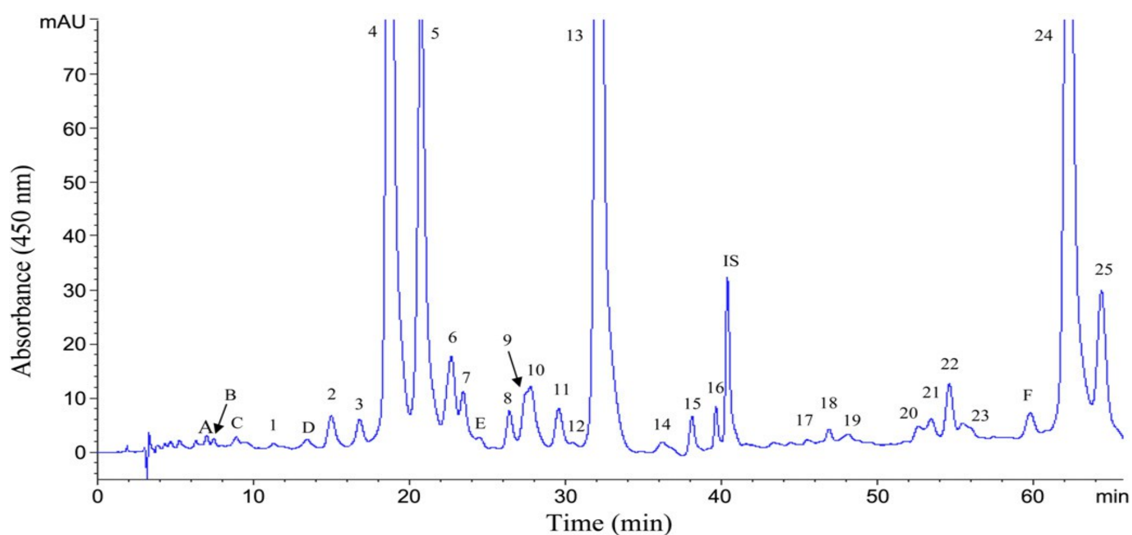

Figure 6. HPLC chromatogram of extract of *T. officinale*.
Peak numbers with alphabetical letters (A-F) indicate additional compounds identified in extract fraction, while 1-25 denote the same standard compound.

This fragmentation indicated the presence of extensive conjugation within the molecule. The ions at m/z 221 and 181 showed that the epoxide group was in a ring with a hydroxyl group. Peaks 17 and 24 were positively identified as all-Trans forms of β-cryptoxanthin and β-carotene, respectively. The mass spectrum of compound 11 identified as lutein (with one b-ring and one e-ring as end-groups), showed fragments at m/z 551 $[M + H-18]^+$, corresponding to the loss of a water molecule (Crupi et al., 2010). Owing to the presence of a double bond allylic to the hydroxyl group, the fragment at m/z 551 is more stable than the protonated molecule (Rivera et al., 2012).The MS^2 spectrum included the fragments $[M + H-18-18]^+$ at m/z 533, $[M + H-18-56]^+$ at m/z 495 and $[M + H-18- 92]^+$ at m/z 459, in agreement with previous studies (Ren et al., 2008). MS analysis of compound 24 showed molecular ion $[M + H]^+$ at m/z 537 and fragment ions MS^2 (m/z 481, 457, 445, 400, 269, 177, 137), and was referred as β-carotene (Dequires et al., 2010). In extracts, the additional peaks A-F were identified as Auroxanthin, 13- cis-neoxanthin, Violaxanthin and 9-cis-β-carotene.

3.4. *Malva neglecta*

Figures 7 & 8 shows the HPLC chromatogram of standards mixture and methanol extract of *Malva neglecta*. ESI–MS^n

mode revealed the presence of peak 1 with molecular ion peak $[M+H]^+$at m/z 191. The major fragments were at m/z 173, 127,109, 85 & 93. The fragment peak with m/z of 173 occurs due to loss of one molecule of water $[M+H-18]^+$. Based on molecular mass and fragmentation pattern peak 1 was tentatively identified as Quinic acid. Peak 2 with molecular ion peak at 133 and m/z of its fragments is 115 & 71. Peak 4 having m/z 169 and fragments m/z as 125 & 79 was identified as Gallic acid. Peak 5 with m/z 353 and m/z of its fragment as 191 identified as Chlorogenic acid. Peak 7 with m/z of 183 and fragmentation m/z as 124 & 78 identified as Tannic acid. Peak 9 with m/z as 151 and its fragments m/z as 136 & 92 identified as vanillin. Peak 10 with m/z 163 with fragments m/z 119 & 93 identified as p- coumaric acid. Peak 12 with m/z 609 and its fragments m/z 300, 271 & 201 was identified as Rutin. Peak 13 m/z 611 and its fragmentation with m/z 303 & 465 identified as Hesperidin. Peaks 15 & 16 were isomers with m/z 137 identified as 4- OH – benzoic acid and salicylic acid having m/z of fragments as 93, 65 & 75. Peak 19 having m/z 147 and its fragments m/z 103, 91 & 77 identified as Coumarin. Peak 20 with m/z 301 and fragments m/z 179,151 & 121 identified as quercetin. Peaks 23 & 24 with m/z 285 having fragments m/z 175, 151 & 121 was identified as luteolin for peak 23. Peak 24 with fragments m/z 217, 133 & 151 was identified as Kaemferol.

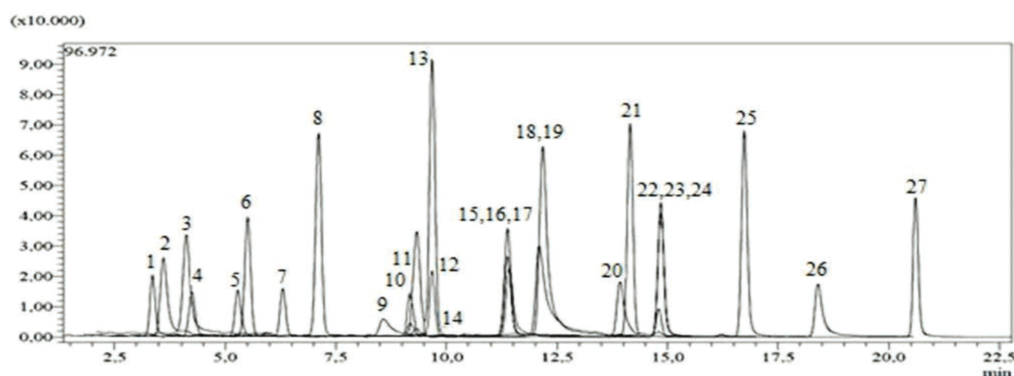

Figure 7. HPLC chromatogram of standards mixture of *Malva neglecta*

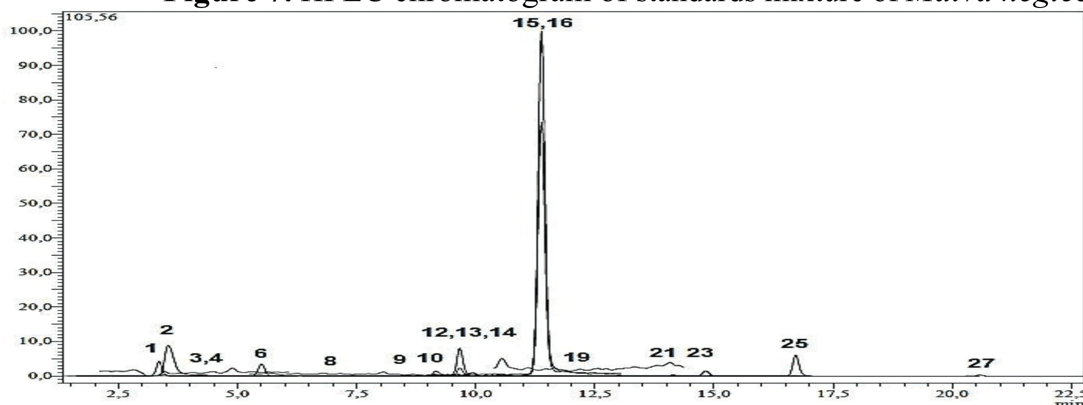

Figure 8. HPLC profile of *Malva neglecta* methanol extract

4.Conclusions

The results from our study, with identified phenolics & carotenoids, together with the generalization of literature data, allowed us to update the knowledge on the phytochemical constituents of *Prunus avium, Cydonia oblonga, Taraxacum officinale* & *Malva neglecta*. Apart of the well-known and largely explored beta-carotene, they contain carotenoids (e.g. lutein, luteoxanthin, antheraxanthin, zeaxanthin) considered as high-value functional products with extensive applications in human affairs.

5. References

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Ethical Approval

This study did not involve any animal or human testing.

Data Availability

No data was used for the research described in the article.