Available on line www.jocpr.com

Journal of Chemical and Pharmaceutical Research

ISSN No: 0975-7384
CODEN(USA): JCPRC5

J. Chem. Pharm. Res., 2011, 3(4):422-429

Separation & identification of simple sugar metabolites from non-edible Pomegranate (*Punica granatum L.*) via TLC and on-line Electrospray Mass Spectrometry

S. Chandraju[1], R. Mythily[1] and C. S. Chidan Kumar[‡]

[1]*Department of Studies in Sugar Technology, Sir M. Vishweshwaraya Post-graduate Centre, University of Mysore, Tubinakere, Mandya, Karnataka, India*
[‡]*Department of Chemistry, Bharathi College, Bharathi Nagar, Karnataka, India*

ABSTRACT

A rapid, sensitive extraction method was developed using the mixture Methanol-Dichloromethane-Water (MDW) (0.3:4:1v/v/v) and MeOH-H_2O phase was assayed for sugar analysis. Photodiode-array detection (DAD) has been used to prove the extracted compound is UV inactive, High-performance liquid chromatography (HPLC) with Evaporative Light Scattering Detector (ELSD) coupled to electrospray ionization mass spectrometric (ESI-MS) detection in the positive ion mode gave MS and MSn fragmentation data which were employed for their structural characterization. The various standard sugars were spotted using the solvent system n-butanol-acetone-pyridine-water (10:10:5:5, v/v/v/v) in the cellulose layer for TLC analysis which indicated the presence of lactose, maltose, glucose, mannose, arabinose. This is the first assay of the sugar profile of the pomegranate skin, which can be further developed for characterization and evaluation of their quality with regards to their sugar composition.

Keywords: Sugar extraction; Pomegranate; UV inactive; HPLC; ELSD; Separation; LC/MS; TLC.

INTRODUCTION

Carbohydrates are among the most abundant compounds in the plant world, and the analysis of sugars and sugar mixtures is of considerable importance to the food and beverage industries[1]. A variety of chromatographic systems including paper and thin-layer chromatography, gas–liquid

chromatography with flame ionization or mass spectrometric detection, and high-performance liquid chromatography (HPLC) can be used to separate and analyze them[1].

The pomegranate tree is native from Iran to the Himalayas in northern India and has been cultivated since ancient times throughout the Mediterranean region of Asia, Africa and Europe. The pomegranate is a shrub, usually with multiple stems, that commonly grows 6-15 ft (1.8-4.6 m) tall. The slender branches start out upright then droop gracefully[2]. Unpruned shrubs have a decidedly weeping or fountain shaped habit. The deciduous leaves are shiny and about 3 in (7.6 cm) long. Pomegranates have beautiful orange-red trumpet shaped flowers with ruffled petals. The flowers are about 2 in (5 cm) long, often double, and are produced over a long period in summer. The pomegranate fruit is globose, 2-3 in (5-7.6 cm) in diameter, and shiny reddish or yellowish green when mature. It has a persistent calyx opposite the stem end that looks like a little crown. The fruit is technically a berry. It is filled with crunchy seeds each of which is encased in a juicy, somewhat acidic pulp that is itself enclosed in a membranous skin. The seeds, juice and pulp are eaten, but the yellowish membrane is too astringent[3].

The juice of wild pomegranates yields citric acid and sodium citrate for pharmaceutical purposes. Pomegranate juice enters into preparations for treating dyspepsia and is considered beneficial in leprosy. The bark of the stem and root contains several alkaloids including *iso*pelletierine which is active against tapeworms. Either a decoction of the bark, which is very bitter, or the safer, insoluble Pelletierine Tannate may be employed. Overdoses are emetic and purgative, produce dilation of pupila, dimness of sight, muscular weakness and paralysis[4]. Because of their tannin content, extracts of the bark, leaves, immature fruit and fruit rind have been given as astringents to halt diarrhea, dysentery and hemorrhages. Dried, pulverized flower buds are employed as a remedy for bronchitis. In Mexico, a decoction of the flowers is gargled to relieve oral and throat inflammation. Leaves, seeds, roots and bark have displayed hypotensive, antispasmodic and anthelmintic activity in bioassay[5]. The present investigation is concentrated mainly on the water soluble sugars present in the peels of pomegranate which have wide application in industries and most economical process.

EXPERIMENTAL SECTION

Selected samples are sliced, dried under vacuum at 60^0C for 48 hr and powdered. 100.0 g of raw material was extracted with doubly distilled water 75mL, 15mL of 0.1N sulphuric acid and kept under hot plate for about 1 hour at 60°C. Contents are cooled and stirred well with magnetic stirrer for 30'. Neutralized using AR barium hydroxide heptahydrate and precipitated barium sulphate is filtered off. The resulting syrup was stored at 4°C in the dark. The syrup was treated with charcoal (coir pith) and agitated for 30' followed by Silica gel (230-400 mesh) packed in a sintered glass crucible for about 2cm thickness connected to suction pump, where rota vapour removed the solvent of the filtrate. The residue was placed in an air tight glass container covered with 200 ml of boiling 80% ethanol. After simmering for several hours in a steam bath, the container was sealed and stored at room temperature. For the analysis, sample was homogenized in a blender for 3-5'at high speed and then filtered through a Buchner funnel using a vacuum source replicated extraction with 80% EtOH (2 x 50mL) each time and the whole syrup was concentrated. Methanol - Dichloromethane - water (0.3:4:1, v/v/v), Sample tubes fed with the mixture were loosely capped, placed in a water bath for 5s, and left at room temperature for

10'and placed in separating funnel, agitated vigorously by occasional release of pressure, results two phases. The organic phase was discarded which removes the organic impurities and the methanol: water phase was assayed for sugar. The residues were oven-dried at 50°C overnight to remove the residual solvent, and stored at –2° C for analysis[6-7].

Instrumentation

The mixture was separated in 26'by reversed phase HPLC on an Adsorbosphere column-NH_2, (250 x 4.6 mm column) using both isocratic and gradient elution with acetonitrile/water and detected using Waters ELSD 2420. In ELSD, the mobile phase is first evaporated. Solid particles remaining from the sample are then carried in the form of a mist into a cell where they are detected by a laser. The separated fractions were subjected to UV analysis using Agilent 8453 coupled with Diode array detector. HPLC–MS analysis was performed with LCMSD/Trap System (Agilent Technologies, 1200 Series) equipped with an electrospray interface. The MS spectra were acquired in positive ion mode. The mobile phase consisted of 0.10% formic acid in hplc grade deionized water (A) (milli-q-water (subjected to IR radiation under 3.5 micron filters) and Methanol (B) taken in the stationary phase of Atlantis dc 18 column (50 x 4.6mm - 5µm). The gradient program was as follows: 10% B to 95% B in 4 min, 95% B to 95% B in 1 min, 95% B to 10% B in 0.5 min followed by 10% B in 1.5 min at a flow rate of 1.2 mL min^{-1}. The column oven temperature was kept at 40°C and the injection volume was 2.0 µL. Product mass spectra were recorded in the range of m/z 150-1000. The instrumental parameters were optimized before the run[6-7].

Preparation of chromatoplates

Thin layer chromatography was performed for the concentrated separated fraction using Cellulose MN 300 G. The fractions obtained were subjected to one dimensional chromatogram on a cellulose layer plate. Each plate was activated at 110°C prior to use for 10'.

Standard samples

Pure samples D (-) Arabinose, D (-) Ribose, D (+) Xylose, D (+) Galactose, D(+) Glucose, D (+) Mannose, L (-) Sorbose, D (-) Fructose, L (+) Rhamnose, D (+) Sucrose and D (+) Maltose, D (+) Lactose were used as standard.

One – dimensional chromatography

10 mg of each sugar and the separated fractions were dissolved in 1ml of deionised water. 1µL of each sugar solution was applied to the chromatoplate with the micropipette in the usual manner. The chromatoplate was placed in the chamber containing the developing solvent. The solvent system used was n-butanol-acetone-pyridine-water (10:10:5:5, v/v/v/v). The plates were developed in an almost vertical position at room temperature, covered with lid [8-11]. After the elution, plate was dried under warm air. The plate was sprayed with 5% diphenylamine in ethanol, 4% aniline in ethanol and 85% phosphoric acid (5:5:1v/v/v). The plate was heated for 10'at 105°C. While drying coloured spots appear. The R_f values relative to the solvent are reported here.

RESULTS AND DISCUSSION

The Mass Spectrum detector gave the following spectrum of fraction1 at 0.578', fraction2 at 0.593', fraction3 at 0.523 and 0.702', fraction4 at 0.547 and 2.572', fraction5 at 0.595 and 2.576'. The MS report recorded at the appropriate time as per MSD for fraction1 scanned between the time period 0.493:0.772' gave m/z values 112.9, 145.1, 163.0, 164.1, 180.1, 202.9. Fraction2 scanned between the time periods 0.507: 0.745' gave m/z values 111.2, 115.1, 140.9, 145.1, 180.1, 198.0, 202.9.

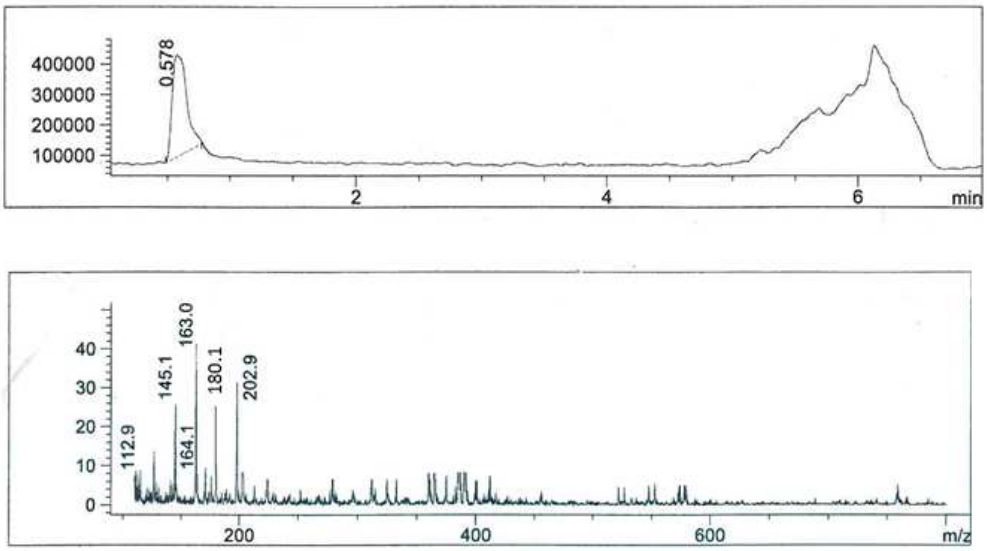

Figure 1: Mass report of Separated Fraction 1

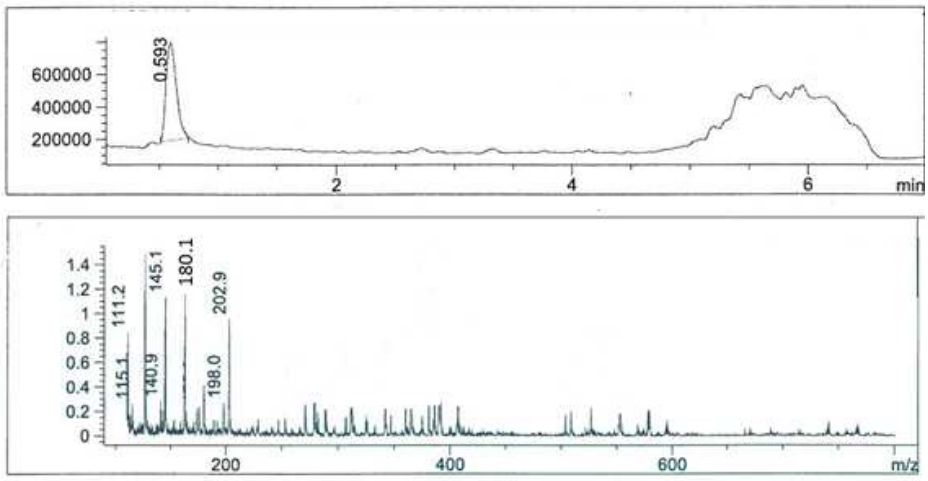

Figure 2: Mass report of Separated Fraction 2

Fraction3 scanned between the time periods 0.480: 0.546 & 0.573: 0.812'gave m/z values 115.1, 145.1, 175.9, 279.2, 312.1, 366.0, 365.0, 707.2 &111.2, 145.1, 279.2, 312.1, 360.0, 365.0, 707.2 respectively. Fraction4 scanned between the time period 0.493:0.600' and 2.482:2.667' gave m/z

values 145.1, 279.2, 312.1, 342.2, 360.0, 366.0, 527.0, 528.0, 689.0 and 112.2, 145.1, 175.9, 278.9, 312.1 respectively.

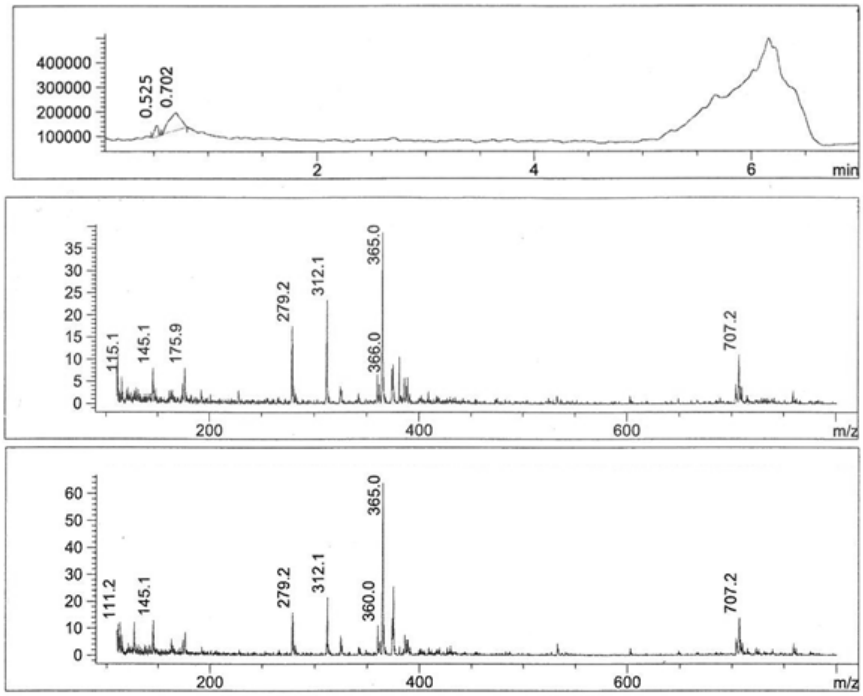

Figure 3: Mass report of Separated Fraction 3

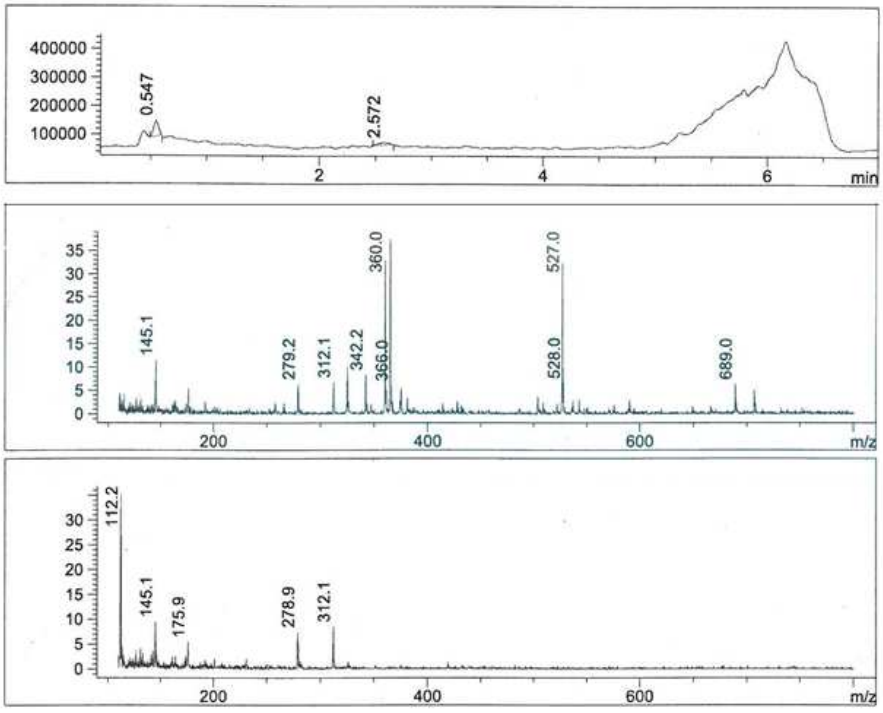

Figure 4: Mass report of Separated Fraction 4

Fraction5 scanned between the time period 0.520 : 0.745 and 2.508 : 2.667' gave m/z values 111.2, 145.1, 150.1, 272.9, 305.1, 326.1, 327.1, 331.0 and 112.2, 145.1, 278.9, 312.1 respectively which gives a conclusion that these masses corresponds to Hexose, pentose and disaccharides whose masses are 180.1, 150.1 and 342.2, 360.0 respectively depicted in Figure 1-5.

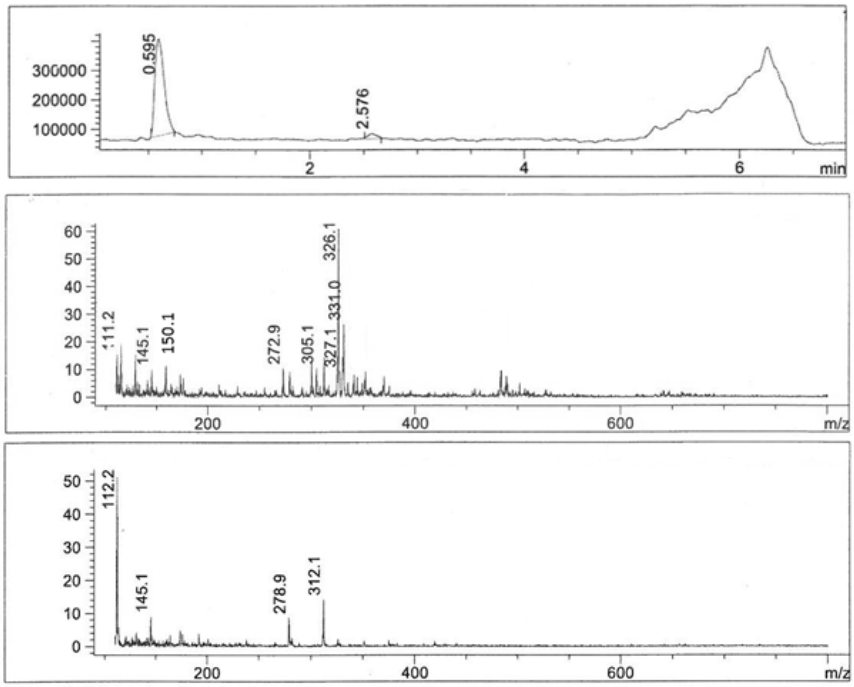

Figure 5: Mass report of Separated Fraction 5

Thin layer chromatographic analysis report

Five separated and purified sample fractions are spotted in the cellulose layer and the eluted species were mentioned as F 1, F 2, F 3, F4 and F5 in the chromatogram shown in Figure 6.

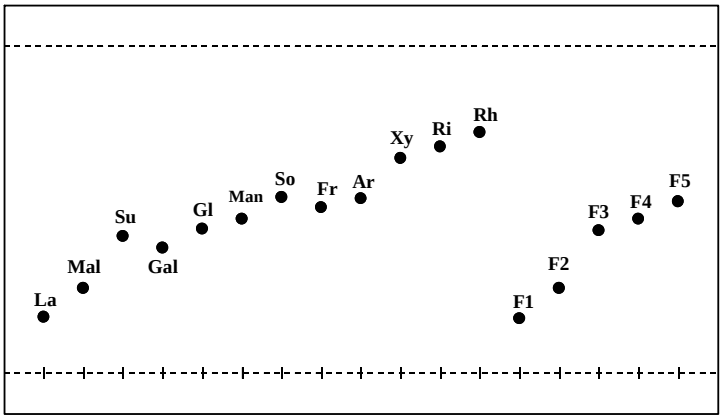

Figure 6: Developed thin layer chromatogram over a cellulose layer, (La – Lactose, So – Sorbose, Ar-Arabinose, Rh – Rhamnose, Ri – Ribose, Xy-Xylose, Gal – Galactose, Gl - Glucose, Man – Mannose, Fr - Fructose, Su – Sucrose and Mal –Maltose).

The fractions obtained were found to be matching with the standard sugars and found to Lactose, glucose maltose, mannose and arabinose. R_f value for the analytical grade samples which also shows the matching fractions Table 1.

Table 1. R_f values matching of the analytical standard samples and the separated samples

Sugars	R_f (Scale of R_f =1)	Fraction matching
Lactose	0.17	F1
Maltose	0.26	F2
Sucrose	0.42	-
Galactose	0.38	-
Glucose	0.44	F3
Mannose	0.47	F4
Sorbose	0.54	-
Fructose	0.51	-
Arabinose	0.53	F5
Xylose	0.66	-
Ribose	0.69	-
Rhamnose	0.74	-

CONCLUSION

The quantity of the discarded portion is very high; therefore, because of disposal problems the household solid wastes are of greater importance. A fruitful and economic industrial application was applied in this current work. Based on the above studies, a rapid method for the extraction of water soluble sugar has been developed. The mixture MDW gives better results as compared with MCW, i.e dichloromethane was replaced instead of chloroform[12]. Mass and TLC analysis gives accurate confirmation for the presence of lactose, glucose, mannose, maltose and arabinose.

REFERENCES

[1] M. Peris-Tortajada in: L.M.L. Nollet (ed.) Handbook of Food Analysis, Marcel Dekker, New York, **1996**; 533–550
[2] Stover E, Mercure EW, *HortScience*, **2007,** 42 (5): 1088–92.
[3] Bialonska D, Kasimsetty SG, Khan SI, Ferreira D, *J Agric Food Chem* **2009**, 57 (21): 10181–6.
[4] Aviram M, Dornfeld L, *Atherosclerosis* **2001**, 158 (1): 195–8.
[5] Menezes SM, Cordeiro LN, Viana GS, *Journal of herbal pharmacotherapy*, **2006**, 6 (2): 79–92.

[6] S. Chandraju, R. Mythily and C. S. Chidan Kumar., *J. Chem. Pharm. Res.*, ***2011***, *3(3):312-321*
[7] S. Chandraju, R. Mythily and C. S. Chidan Kumar., "Liquid Chromatography/Mass pectroscopy and TLC analysis for the identification of sugars extracted from the outer skin of Almond fruit (Prunus dulcis)". *Recent Research in Science and Technology*, In press
[8] Baldwin, E;, Bell, D.J.; *Cole's Practical Physiological Chemistry*, published by Heffer, Cambridge, **1955**, 189.
[9] A .; Schweiger, *Journal of Chromatography*, **1962**, 9, 374.
[10] D. W.;Vomhot, T. C.; Tucher, *Journal of Chromatography* **1963**, 17, 300.
[11] M.; Lato, Brunelli, B.; G.; Ciuffins. *Journal of Chromatography*, **1968**, 34, 26.
[12] Pak S.; Chow, Simon M.; Landhäusser. *Tree Physiology* **2004**, 24, 1129 –1136