Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2012, 4(4):1631-1636
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

A rapid and sensitive extraction of sugars from papaya peels (*Carica Papaya*)

C. S. Chidan Kumar*[1], R. Mythily[2] and S. Chandraju[2]

[1]*Department of Chemistry, Alva's Institute of Engineering & Technology, Shobhavana Campus, Mijar, Moodbidri 574 225, South Canara District, Karnataka, India.*
[2]*Department of Studies in Sugar Technology, Sir M. Vishweshwaraya Post-graduate Center, University of Mysore, Tubinakere, Mandya-571 402, Karnataka, India.*

ABSTRACT

Papayas have been part of human diet for ages due to their health benefits. But consumption of these fruits generates outer skin wastes that may lead to environmental pollution. This study was carried out to explore the sugar components of papaya peels. Selected samples are cut into small bits, dried, powdered and were subjected to sensitive extraction procedure developed using the mixture Methanol –Dichloromethane - Water (MDW) (0.3:4:1v/v/v) and MeOH-H_2O phase was assayed for sugar analysis. The extracted sugars were put through some chemical characterization procedures for purposes of separation and identifying its components. The various standard sugars were spotted using the solvent system n-butanol-acetone-pyridine-water (10:10:5:5, v) in the cellulose layer for TLC analysis which indicated the presence of glucose, maltose, fructose.

INTRODUCTION

Carica papaya contains an enzyme known as papain, present in the fruit, stem and leaves [1]. The milky juice is extracted, dried and used as chewing gum, medicine (digestion problems), and toothpaste and meat tenderizers [2]. Meat can be tenderized by wrapping it in a bruised papaya leaf before it is cooked. Carica papaya contains many biologically active compounds. Two important compounds are chymopapain and papain, which are supposed to aid in digestion[3]. Papain also is used to treat arthritis. The level of the compounds varies in the fruit, latex, leaves, and roots. Papaya has been used for digestive problems and intestinal worms. The softening and disintegrating qualities of papain (generally in alkaline combination, as with borax or potassium carbonate), have been taken advantage of in the treatment of warts, corns, sinuses, and chronic forms of scaly eczema, cutaneous tubercles, and other hardness of the skin, produced by irritation, etc., [4] and injected into indolent glandular tumors to promote their absorption. Green fruits are used to treat high blood pressure and also used as an aphrodisiac. It is useful in round worm infestation, stomachalgia, dyspepsia, constipation, amenorrhoea, skin diseases and general debility [5].

Inspite of all these advantages t consumption of these fruits generates outer skin wastes that could bring about environmental pollution if not properly handled. Towards recycling of vegetative wastes avoiding littering and waste-related environmental degradation, this study was carried out to explore the sugar components of papaya peels with a view to establishing their raw material potentials.

Moreover the basic liquid-liquid sugar extraction procedure was successful with various peels such as banana, pomegranate, custard apple, pine apple, almond fruit, orange, calabash and musk melon *etc*., [6-16] it created the interest to explore sugar components from papaya peels too.

MATERIALS AND METHODS

Instrumentation

An HPLC system equipped with Waters 2420 Evaporative Light Scattering Detector (ELSD) is used to separate the fractions from the extract. An LCMSD/Trap System (Agilent Technologies, 1200 Series) equipped with an electro spray interface was used in the second step. An Agilent 8453 spectrophotometer coupled with a diode array detector was used for the detection of sugars in the fractions. Two chromatographic columns used were: an Adsorbosphere-NH_2 column (250 x 4.6 mm-5µm) and an Atlantis dc 18 column (50 x 4.6mm - 5µm).

Materials

All reagents and solvents used were of HPLC grade. Formic acid, methanol, acetonitrile, dichloromethane and ethanol were from Fisher Scientific Co. HPLC water prepared from distilled water using a Milli-Q system (Millipore Lab., Bedford) was subjected to IR irradiation under 3.5 micron filters. All chemicals used in the study were of analytical grade.

Extraction

Selected samples of water melon were sliced, dried under vacuum at 60°C for 48 h and powdered. 100.0 g of the raw material was extracted with doubly distilled water (75 mL) and 0.1N sulphuric acid (15 mL) and kept at 60°C for about 5 h. Contents were cooled and stirred with magnetic stirrer for 30 min. The extract was treated with calcium carbonate as such (2-3g) and the precipitated calcium sulphate was filtered off. The resultant syrup was stored at 4°C in the dark. The syrup was treated with charcoal (coir pith), agitated for 30 min and suction filtered using a sintered glass crucible packed with Silica gel (230-400 mesh, ~2 cm thickness). The filtrate was rota vapoured to remove the solvent. The residue was placed in an air tight glass container covered with 200 ml of boiling 80% EtOH. After simmering for several hours in a steam bath, the container was sealed and stored at room temperature. For the analysis, the sample was homogenized in a blender for 3-5min at high speed and then suction-filtered. After extraction with 80% EtOH (2 x 50mL), the whole syrup was concentrated and further extracted in a separator funnel using a mixture of MeOH - dichloromethane - water (0.3:4:1, v/v/v), The organic phase containing the organic impurities was discarded and the MeOH- water phase containing sugars was evaporated. The residue was oven-dried at 50°C overnight to remove the residual solvent and stored at –2°C for chromatographic analysis [6-16].

HPLC and LC/MS

The extracted sugars present in the non-edible watermelon were separated in 26 min by the reversed phase HPLC technique on a column, Adsorbosphere-NH_2 (250 x 4.6 mm column) using both isocratic and gradient elution with acetonitrile/water as the mobile phase. The detector used was a Waters ELSD 2420. In the ELSD detector, the mobile phase is first evaporated and the remaining solid particles of 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 spectrophotometer coupled with a diode array detector. The LC/MS analysis was performed with LCMSD/ Trap System (Agilent Technologies, 1200 Series) equipped with an electro spray interface. The mobile phase consisted of 0.10% formic acid in HPLC grade de-ionized water (A) 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.

Standard Samples

Pure samples of 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 standards (Aldrich Chemical Co.).

Preparation of Chromatoplate

The sugar fractions separated from HPLC were concentrated and subjected to thin-layer chromatography (TLC). The sugar fractions were analyzed with one-dimensional TLC on a cellulose MN 300 G layer plate. Each plate was activated at 110°C prior to use for 10 min.

One Dimensional Chromatography

One mL aqueous solution of 10-mg sample of each standard sugar or the analyzed sugar from an HPLC fraction was prepared. After 1 µL of each sugar solution was applied, the chromatoplate was placed in a TLC chamber containing the developing solvent and the lid placed. 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 [17-20]. After the elution, plate was dried under warm air. The plate was sprayed with a mixture of 5% diphenylamine in EtOH, 4% aniline in EtOH and 85% phosphoric acid (5:5:1v/v/v). The plate was heated for 10 min at 105°C to visualize colored spots and calculate R_f values.

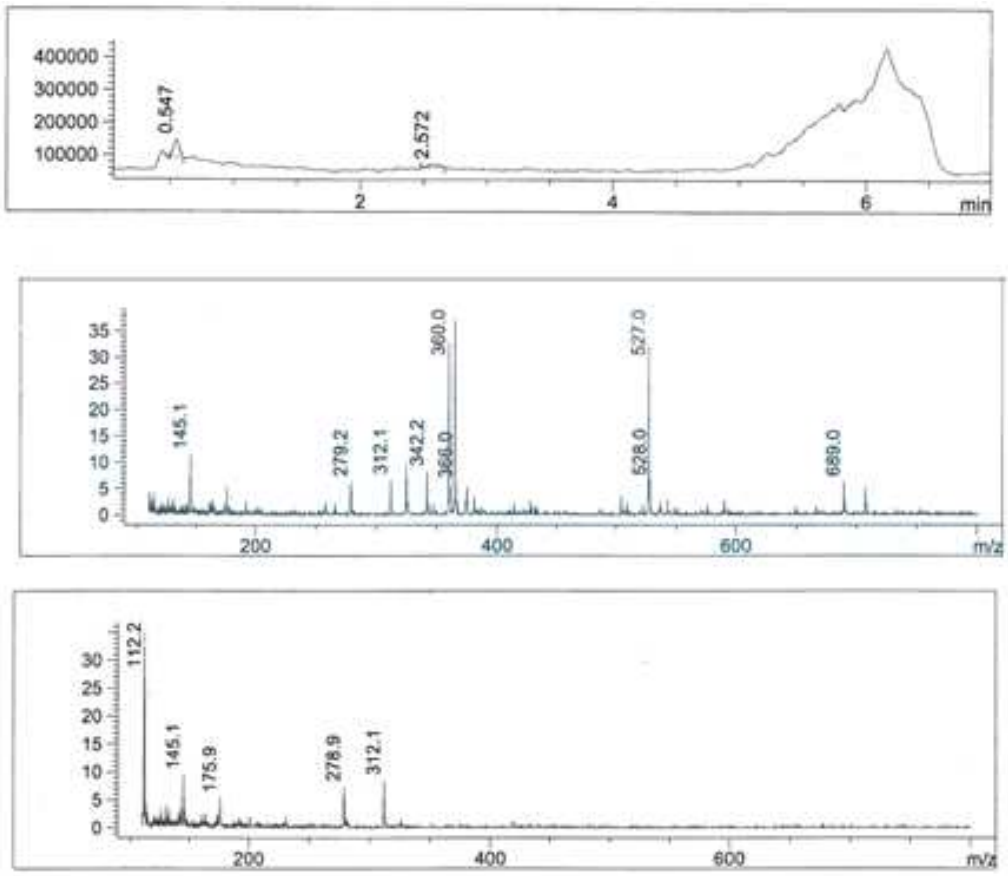

FIGURE 1: Mass report of Separated Fraction 1

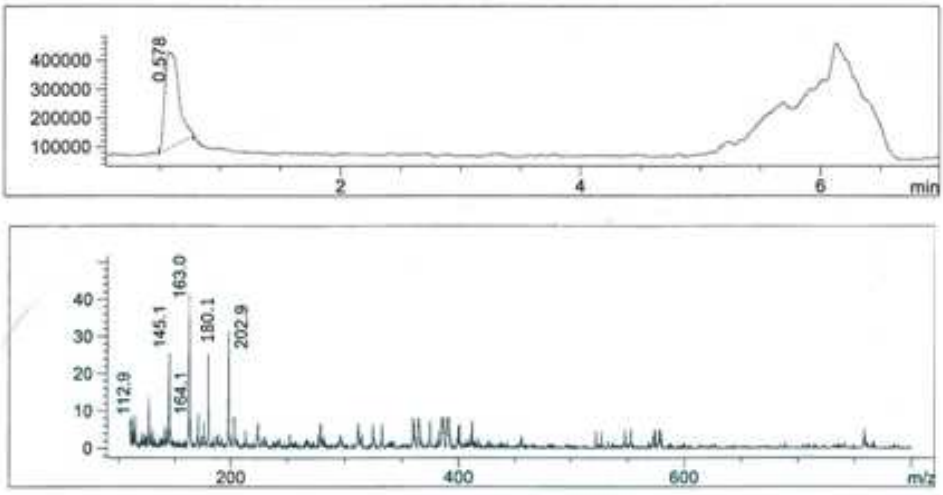

FIGURE 2: Mass report of Separated Fraction 2

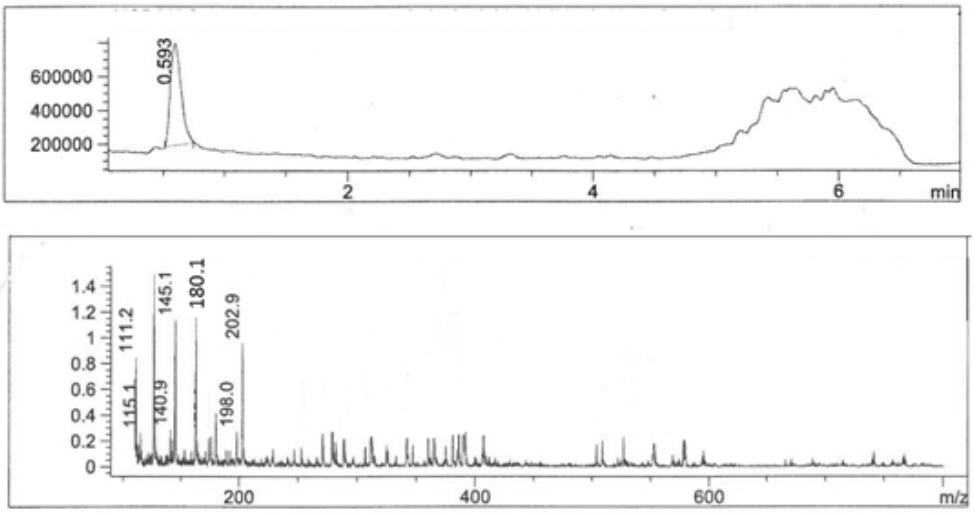

FIGURE3: Mass report of Separated Fraction 3

Mass Spectral Analysis

FIGURE1-3 shows the following LC peaks: fraction1 at 0.547 min and 0.572 min; fraction2 at 0.578 min; and fraction3 at 0.593 min; The LC-MS data for fraction1 scanned in the time periods 0.493:0.600 min and 2.482:2.667 min show molecular ion fragments at m/z: 112.2, 145.1, 175.9, 278.9, 279.2, 312.1, 342.2, 360.0, 366.0, 528.0, 527.0, 689.0 respectively. Fraction2 scanned between the time periods 0.493:0.772 min gave ion peaks at m/z: 112.9, 145.1, 163.0, 164.1, 180.1, and 202.9. Fraction3 scanned between the time periods 0.493:0.745 min gave ion peaks at m/z: 111.2, 115.1, 140.9, 145.1, 180.1, 198.0 and 202.9. These mass data lead to the conclusion that there are various monosaccharide and disaccharides.

Thin-layer chromatographic analysis

Sugars obtained from four HPLC fractions of the watermelon peels were spotted on the cellulose layer of TLC plates and the eluted compounds were labeled as F1, F2 and F3 on the chromatogram as shown in FIGURE 4. The R_f values of analyte sugars from fractions were found to match with the R_f values of authentic samples of sugars leading to the identification of the former as maltose, glucose and fructose. Table 1 shows R_f for the standard sugars and the matching analyte sugars extracted from papaya peels.

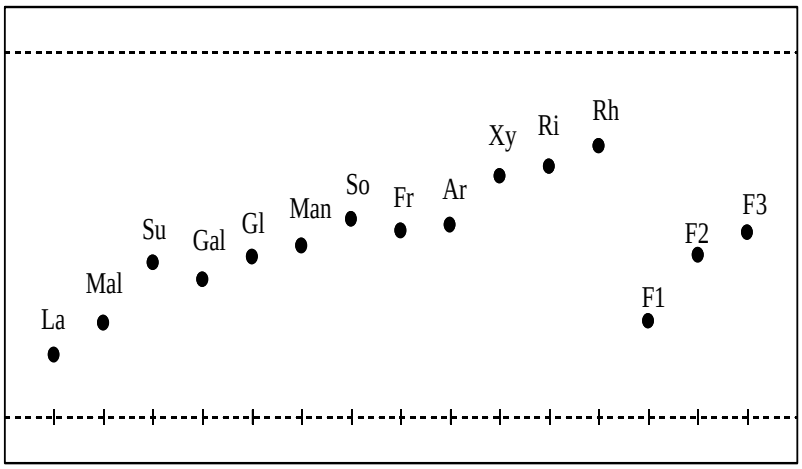

FIGURE 4: Developed thin layer chromatogram over a cellulose layer, (La – Lactose, So – Sorbose, Ar- Arabinose, Rh – Rhamnose, Ri – Ribose, Xy-Xylose, Gal – Galactose, Gl - Glucose, Man – Mannose, Fr - Fructose, Su – Sucrose and Mal –Maltose).

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	-
Maltose	0.26	F1
Sucrose	0.42	-
Galactose	0.38	-
Glucose	0.44	F2
Mannose	0.47	-
Sorbose	0.54	-
Fructose	0.51	F3
Arabinose	0.53	-
Xylose	0.66	-
Ribose	0.69	-
Rhamnose	0.74	-

REFERENCES

[1] O. Oderinde, C. Noronha, A. Oremosu, T. Kusemiju, and O. A. Okanlawon, *Niger. Postgrad. Med. J*, **2002**, 9(2), 95–98.
[2] N. K. Lohiya, B. Manivannan, P. K. Mishra, N. Pathak, S. Sriram, S. S. Bhande, and S. Panneerdoss, *Asian J Androl*, **2002**, 4(1), 17–26.
[3] J. F. Morton, *Papaya. In: Fruits of warm climates*. **1987**, 336–346.
[4] F. Echeverri, F. Torres, W. Quinones, G. Cardona, R. Archbold, J. Roldan, I. Brito, and J. G. Luis, *Phytochemistry*, **1997**, 44(2), 255-256.
[5] V. P. Titanji, D. Zofou, and M. N. Ngemenya, *Afr. J. Tradit. Complement. Altern. Med*, **2008**, 5(3), 302–321.
[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, *J. Chem. Pharm. Res*, 3(4), 422-429.
[8] S. Chandraju, R.Mythily, and C.S. Chidan Kumar, *J. Chem. Pharm. Res*, vol. 4, no. 2, pp. 1312-1318, **2012**.
[9] S. Chandraju, R.Mythily, and C. S. Chidan Kumar, *Int J Cur Sci Res*, **2011**, 1(3), 125-128.
[10] S. Chandraju, R. Mythily, and C. S. Chidan Kumar, *Rec Res Sci Tech*, **2011**, 3(7), 58-62.
[11] C. S. Chidan Kumar, R. Mythily, and S. Chandraju, *Asian J. Chem*, **2011**, 24(5), 2170-2172.
[12] C. S. Chidan Kumar, R. Mythily, and S. Chandraju, *Biosci. Biotech. Res. Asia*, **2011**, 8(2), 709-715.
[13] C. S. Chidan Kumar, R. Mythily, and S. Chandraju, *Int J Cur Chem Sci*, **2011**, 1(1), 008-011.
[14] C. S. Chidan Kumar, R. Mythily, and S. Chandraju, *Int.J. ChemTech Res*, **2012**, 4(1), 438-444.
[15] R. Mythily, C. S. Chidan kumar, S. Chandraju, *Int J Chem Res*, **2012**, 3(1), 40-44.
[16] C. S. Chidan Kumar, R. Mythily, and S. Chandraju, *Int J Cur Res and Rev*, **2011**, 3(2), 201-206.
[17] E. Baldwin, and D. J. Bell, "Cole's Practical Physiological Chemistry," **1955**, 189.

[18] A. Schweiger, *J Chromatography A,* **1962**, 9, 374.
[19] D. W. Vomhot, and T. C. Tucher, *J Chromatography A***, 1963**, 17, 300.
[20] M. Lato, B. Brunelli and G. Ciuffins, *J Chromatography A***, 1968**, 26, 34.