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EXTRACTION AND MASS CHARACTERIZATION OF SUGARS FROM ASH GOURD PEELS (*Benincasa Hispida*)

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ABSTRACT

Selected samples of Ash gourd peels are cut into small bits, dried, powdered and subjected to sensitive extraction procedure developed using the mixture of 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/v/v/v) in the cellulose layer for TLC analysis which indicated the presence of Galactose, Glucose, Xylose, Sorbose.

Keywords: Sugars, ash gourd, Separation, Identification, Waste Management.

INTRODUCTION

Ash gourd is actually a fruit but is referred to as a vegetable because it is cooked and eaten as a vegetable. It is also known as white gourd, winter melon, white pumpkin, wax gourd etc. It is oblong in shape, and 1 to 2 metres in length, it has a smooth rind that is an ashy green with white flesh, and big, flat, oval seeds. These gourds have been cultivated since ancient times in India, Malaysia, and China etc. In India, the ash-gourd is offered to the gods in religious ceremonies[1]. What contributes to its longevity is the chalky wax on its skin which prevents micro-organisms from attacking it and preserves it. Ash-gourd is enriching with nutrients. It is an excellent source of vitamin B1 (thiamine) and vitamin B3 (niacin), and vitamin C. It is also rich in calcium and potassium, this a good vegetable for maintaining a healthy blood pressure. In Ayurveda, ash-gourd is also used to treat mental illnesses and nervous disorders such as epilepsy, paranoia, and insanity. Ash-gourd is alkaline in nature and hence has a cooling and neutralizing effect on stomach acids and as such used effectively for treating digestive ailments like hyperacidity, dyspepsia, and ulcers[2]. It is also used to treat diabetes.

Ash-gourd is also useful in treating respiratory disorders like asthma, blood-related diseases, and urinary diseases like kidney stone. Every part of this fruit is useful. Ash-gourd leaves are rubbed on bruises to heal them, while the seeds are used for expelling intestinal worms. The ash made from burning the rind and seeds are mixed with coconut oil and used to promote hair growth and to treat dandruff. Inexpensive and versatile, a healthful vegetable that should definitely be a part of any nutritious diet[3]. Thus ash gourd has been part of human diet for ages due to its nutritional and medicinal values. But consumption of these fruits generates peel wastes that could bring about environmental pollution if not properly handled. Towards recycling of wastes and avoiding littering and waste-related environmental degradation, this study was carried out to explore the sugar components of ash gourd peels with a view to establishing their raw material potentials[4]. To our knowledge, this is the first time these sugars are analyzed from the peels of ash gourd. This study forms a part of a series of investigations that were carried out in our laboratory to understand sugar profiles in the peels of several fruits including pomegranate, pineapple, banana, black grape, and almond [5-15].

EXPERIMENTAL

Selected samples are sliced, dried under vacuum at 60°C for 48 h 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 5 h at 60°C. Contents are cooled and stirred well with magnetic stirrer for 30'. Neutralized using AR barium hydroxide 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.

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 electro spray interface. The MS spectra were acquired in positive ion mode. The mobile phase consisted of 0.10% formic acid in HPLC grade deionised 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.

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). The plates were developed in an almost vertical position at room temperature, covered with lid [16-19]. 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 below.

RESULTS AND DISCUSSION

The Mass Spectrum detector gave the following spectrum of fraction1 at 0.606 and 2.637min, fraction2 at 0.578 min, fraction3 at 0.584min and fraction4 at 0.437 and 0.572 min. The MS report recorded at the appropriate time as per MSD for Fraction1 scanned between the time period 0.507: 0.798min gave m/z values 112.9, 145.1, 163.0, 180.1, 198.0, 360.0 and2.495: 2.760min gave m/z value 112.1. Fraction 2 scanned between the time periods 0.493:0.772min gave m/z values 112.9, 145.1, 163.0, 164.1, 180.1, 202.9. Fraction 3 scanned between 0.520:0.745' gave m/z values 145.1, 150.1. Fraction4 scanned between the time periods 0.387: 0.493min gave m/z values 115.1, 140.9, 143.1, 160.9, 173.1, 199.1.230.9 and 0.520:0.758 min gave m/z value 126.9, 163.0 and 202.9. which gives a conclusion that these masses corresponds to Hexose, pentose and disaccharides whose masses are 180.1, 150.1 and 199.1 respectively (Fig. 1-4)

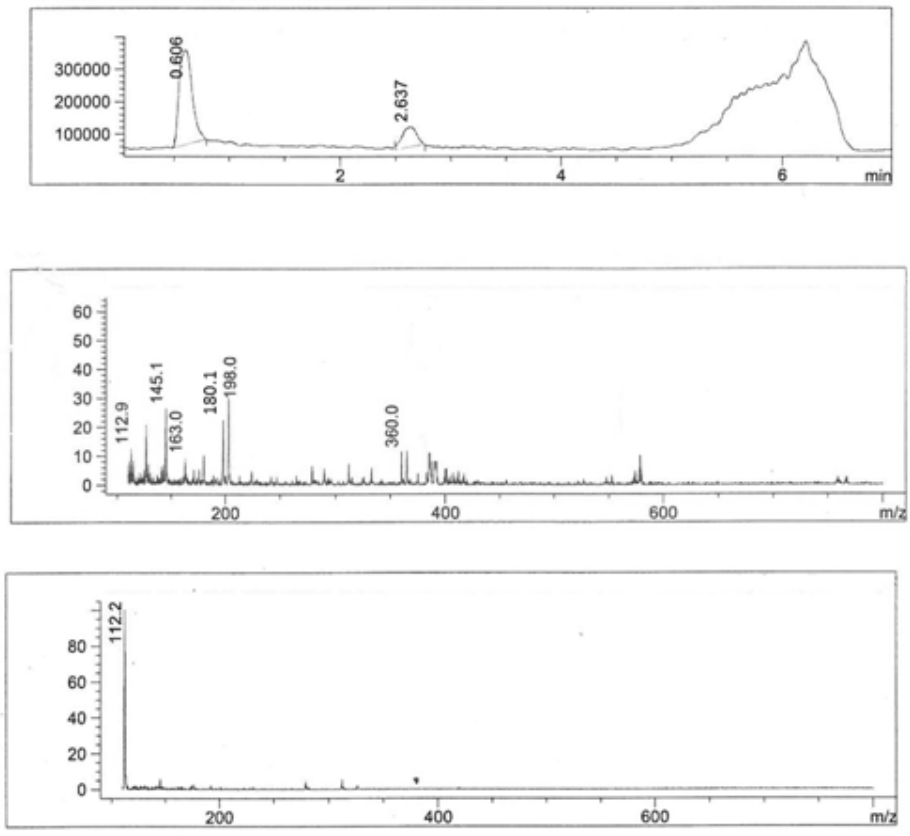

Fig.-1: Mass Spectra of Separated Fraction 1

Thin layer chromatographic analysis report

Four separated and purified sample fractions are spotted in the cellulose layer and the eluted species were mentioned as F1, F2, F3 and F4 in the chromatogram shown in Figure 5. The fractions obtained were found to be matching with the standard sugars and found to Galactose, glucose, Sorbose and xylose. R_f value for the analytical grade samples which also shows the matching fractions Table-1.

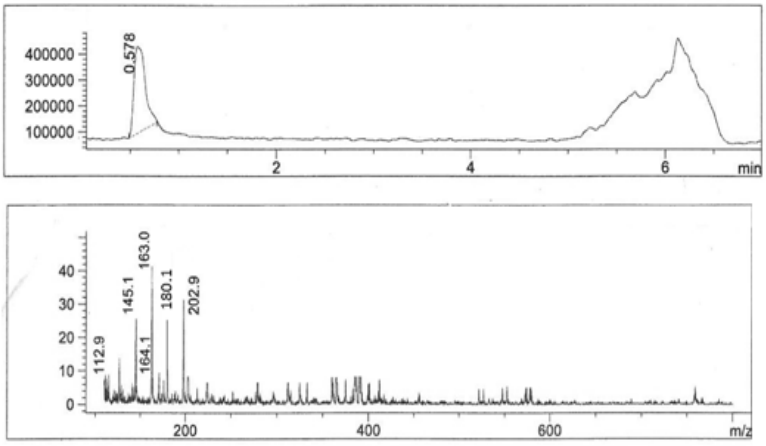

Fig.-2 Mass Spectra of Separated Fraction 2

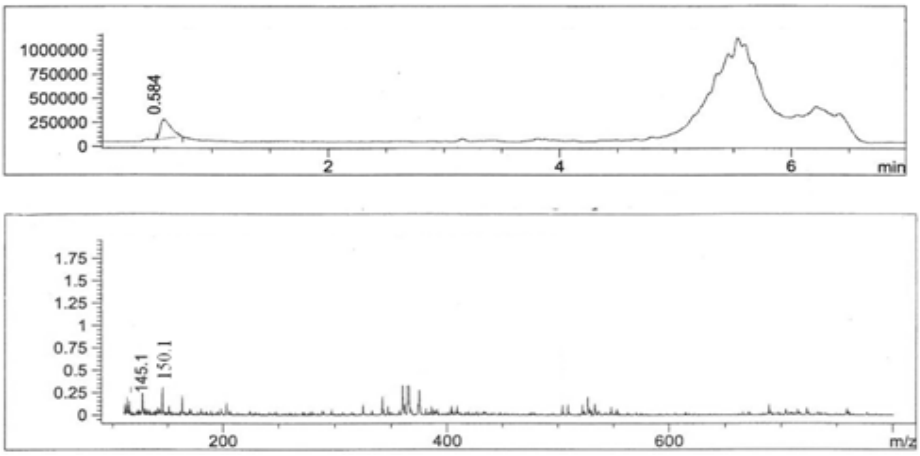

Fig.-3 Mass Spectra of Separated Fraction 3

Table-1

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

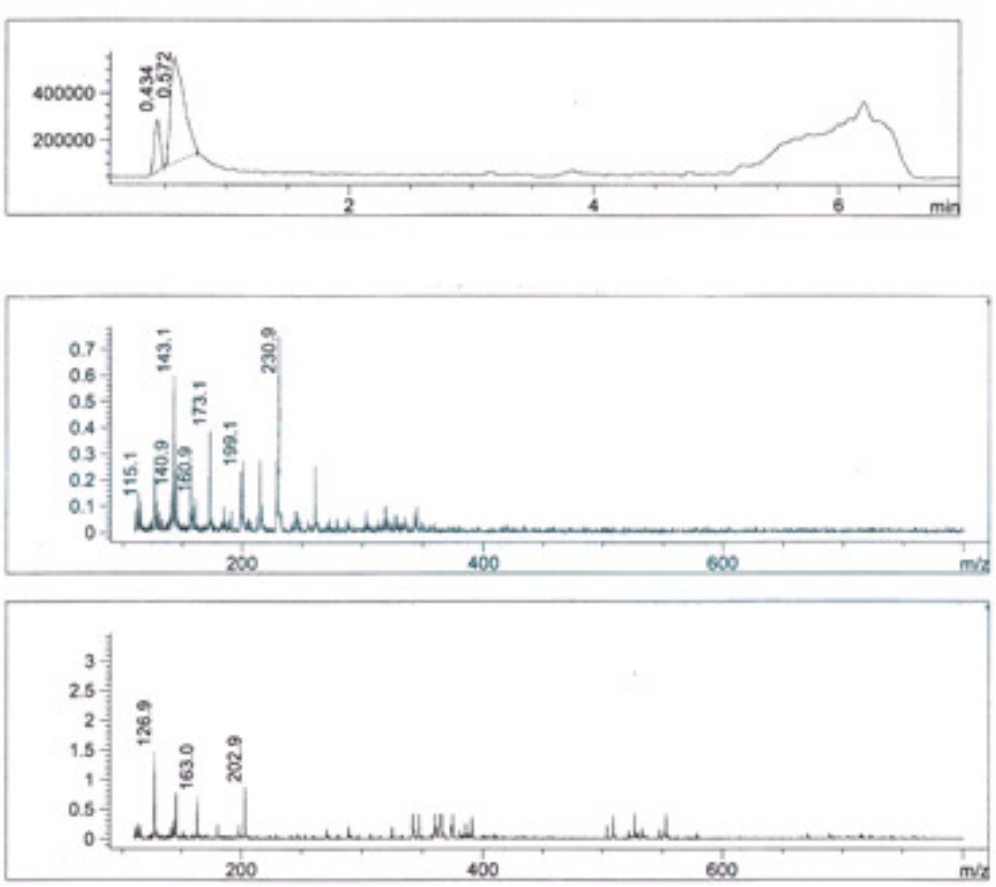

Fig.-4 Mass Spectra of Separated Fraction 4

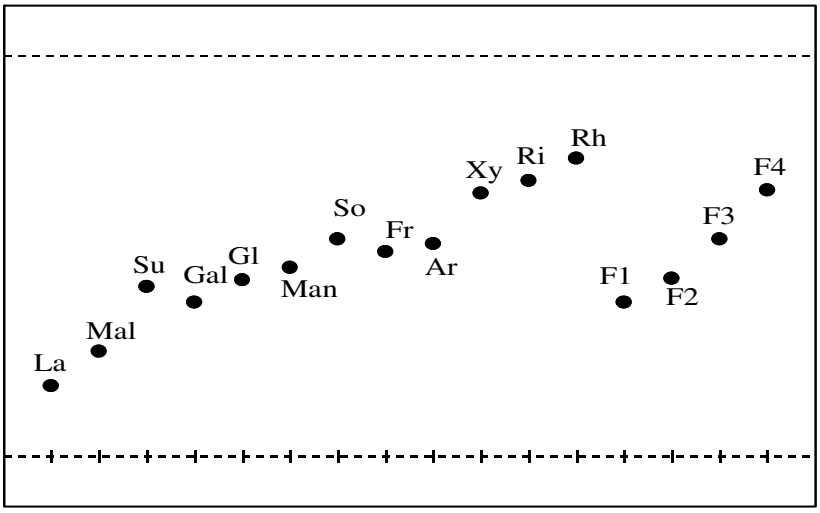

Fig.-5 Developed thin layer chromatogram over a cellulose layer, (La – Lactose, So – Sorbose, Ar- Arabinose, Rh – Rhamnose, Ri – Ribose, Xy-Xylose, Gal – Galactose, Gl - Glucose, Man – Mannose, Fr - Fructose, Su – Sucrose and Mal –Maltose).

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