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Extraction, Isolation and Identification of Sugars from Banana peels (*Musa Sapientum*) by HPLC coupled LC/MS instrument and TLC analysis

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ABSTRACT

The production of various sugars evaluated from the non edible (peel) portion of banana fruit has considerable promise in the future to achieve economical profit. Selected samples were dried, powdered and sequentially extracted with double distilled water, 80% ethanol with replicates and finally with the mixture of Methanol - Dichloromethane - Water (MDW) (0.3:4:1v/v/v). MeOH-H_2O phase was assayed for sugar analysis. The concentrated compound was UV inactive and thus the compound is subjected to HPLC coupled ELSD gave a merged single ionised peak at R_t = 0.646. These crude mixtures are subjected to preparative HPLC coupled LC/MS which gives four compounds whose retention time were found to be 0.662, 0.676, 0.652, 0.684 and with corresponding mass fragments. The various standard sugars were spotted using the solvent system n-butanol - acetone - diethylamine - water (10:10:2:6 v/v/v/v) in the cellulose layer for TLC analysis which indicated the presence of glucose, fructose, sucrose and maltose.

Keywords: Sugar extraction; Banana peels; UV inactive; HPLC; ELSD; Isolation; LC/MS; TLC.

INTRODUCTION

Musa sapientum is commonly called banana is an herbaceous plant of the family *Musaceae*. It is known to have originated from the tropical region of Southern Asia. It is now cultivated throughout the tropics [1]. Plant is cultivated primarily for its fruits and to a lesser extent for the production of fibre [2]. It is also used as an ornamental plant. The *Musa sapientum* grows up to a height of about 2-8m with leaves of about 3.5m in length. The stem which is also called pseudostem produces a single bunch of banana before dying and replaced by new pseudostem. The fruit grows in hanging cluster, with about twenty fruits to a tier and 3 – 20 tiers to a bunch.

The fruit is protected by its peel which is discarded as waste after the inner fleshy portion is eaten. *Musa sapientum* fruits have been reported to prevent anemia by stimulating the production of haemoglobin in the blood. Its role to regulate blood pressure has been associated with the high content of potassium [2]. Banana helps in solving the problem of constipation without necessary resorting to laxatives. Banana can cure heart burns stress, strokes, ulcers and many other ailments [3]. The peels have been reported to be useful in making banana charcoal, an alternative source of cooking fuel in Kampala. The peels in conjunction with other substances create a liniment for reducing the acuteness of the arthritis aches and pains [4]. Considering the upsurge in the prizes of commercial sugars and their increasing demand, this study was conducted to provide information about the *Musa sapientun* peel which is often ignore and considered as waste could be domesticated for proper utilization in an economical manner.

EXPERIMENTAL SECTION

Extraction of water soluble sugars from the samples

Selected samples were sliced, dried under vacuum at 60^0 C for 48 hr, blended to get fine powder, since surface area facilitates the reaction rate. Powdered sample was used for further analysis. 100.0 g of the raw material was taken in 250mL beaker with 50mL of double distilled water, stirred well using a magnetic stirrer for 30'. All soluble sugars dissolved at this stage with other water soluble impurities (Each extraction was replicated four times). The resulting syrup was immediately stored at 4°C in the dark. Coir pith is burnt in presence of air. The black residue obtained was cooled and finely grounded. The syrup was treated with this charcoal and agitated using shaker for about 30'. Adsorption of the coloring materials takes place over the surface of the charcoal. Filtrate was collected. Silica gel (230-400 mesh) was packed in a sintered crucible for about 2cm and the filtrate was poured into the packed fraction in minimal quantity connected to suction pump which brings high vacuum. The coloured impurities are adsorbed and retained with silica gel, the filtrate was collected and solvent was removed in rotating evaporator. The residue was placed in a glass air tight container and covered with 300 ml of boiling 80% ethanol. After simmering for several hours in a steam bath, the container was sealed and then 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. The residue in the funnel was extracted twice (again), using 150 ml of 80% ethanol each time. The extracts were then combined and volume was concentrated in a rotating evaporator for removal of alcohol. MDW in the ratio (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 taken in separating funnel, agitated vigorously by occasional release of pressure, two phases separated. 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.

Preparative HPLC Analysis

The crude sample mixture was subjected to Preparative HPLC for separation into various fractions. 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.

UV Analysis
Crude sample and the separated fractions were subjected to UV analysis using Agilent 8453 coupled with Diode array detector.

HPLC/LC/MS Analysis
The extracted product was analyzed using an LC/MS technique performed on an HPLC system with an Evaporative Light Scattering Detector (Waters 2420) with analog – digital convertor (ADC), as from UV analysis the sample found to be UV inactive which is coupled to a mass spectrometer (Agilent Technologies, 1200 Series) with a mass spectrum detector (MSD). The mobile phase consisted of 0.10% formic acid in HPLC grade deionised water (A) (milli-q-water (subjected to IR radiation under 3.5micron filter) 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 (-) Ribose, D (+) Xylose, D (+) Galactose, (+) Glucose, D (+) Mannose, D (-) Fructose, D (+) Sucrose and D (+) Maltose 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 - diethylamine - water (10:10:2:6 v/v/v/v). The plates were developed in an almost vertical position at room temperature, covered with lid [5-9]. 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 colored spots appear. The R_f values relative to the solvent are reported above.

RESULT AND DISCUSSION

UV report of crude and separated fractions
Analysis report showed that the sample under analysis is UV inactive (No conjugation). UV Spectrum for crude sample was given under Figure 1, and separated fraction 1,2,3,4 (Figures 2,3, 4 and 5).

HPLC report of crude and separated fractions
HPLC reports of crude gave a merged single ionised peak at the R_t = 0.646 shown under Figure 6 and the separated fractions 1,2,3,4 gave a sharp single ionised peak at the R_t = 0.662, 0.676, 0.652, 0.684 respectively (Figures 7, 8, 9 and 10).

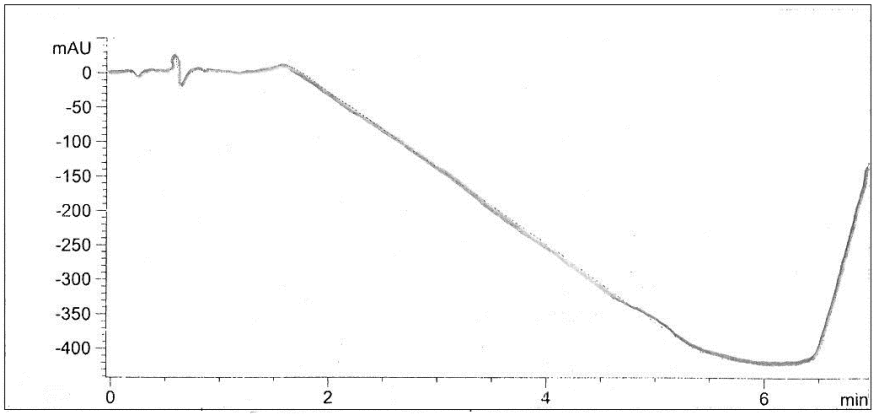

Figure 1. UV Report – Crude Mixture

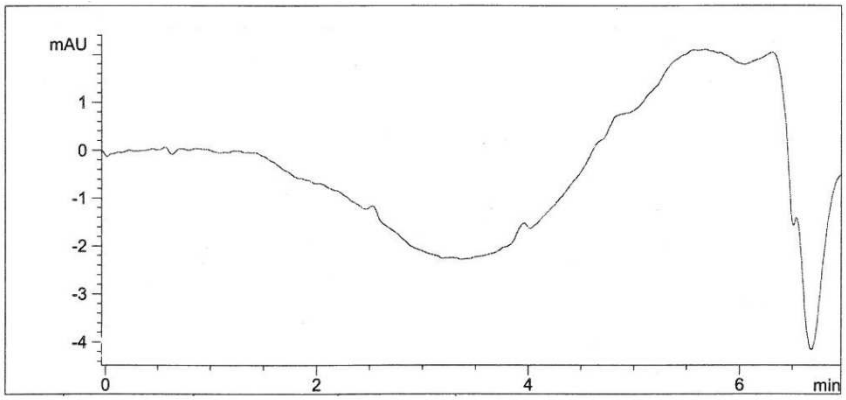

Figure 2. UV Report – Fraction 1

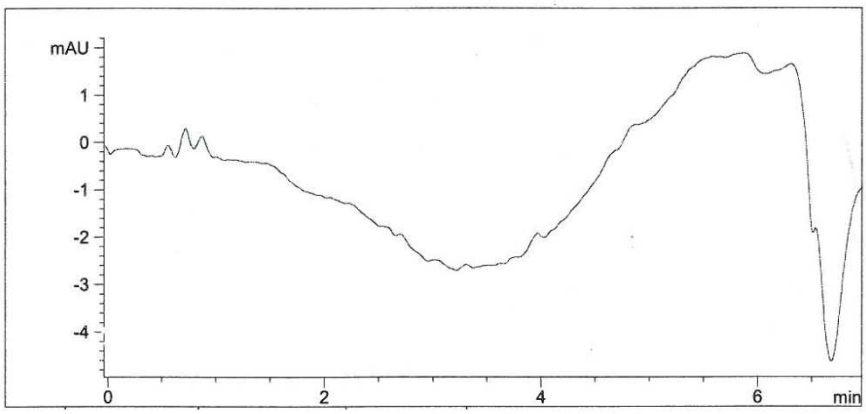

Figure 3. UV Report – Fraction 2

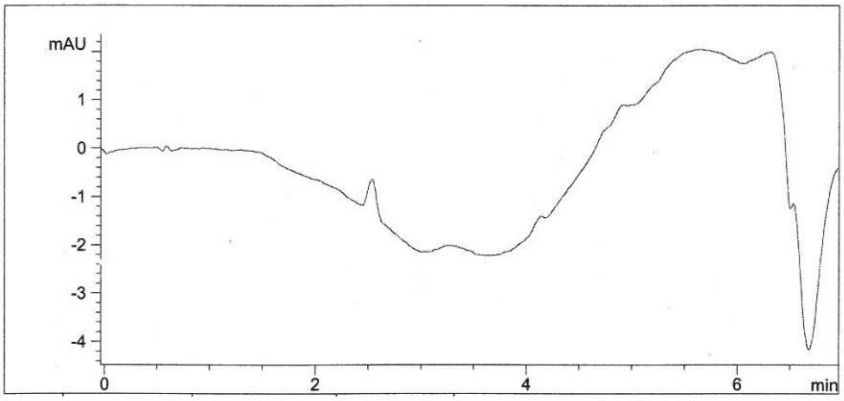

Figure 4. UV Report – Fraction 3

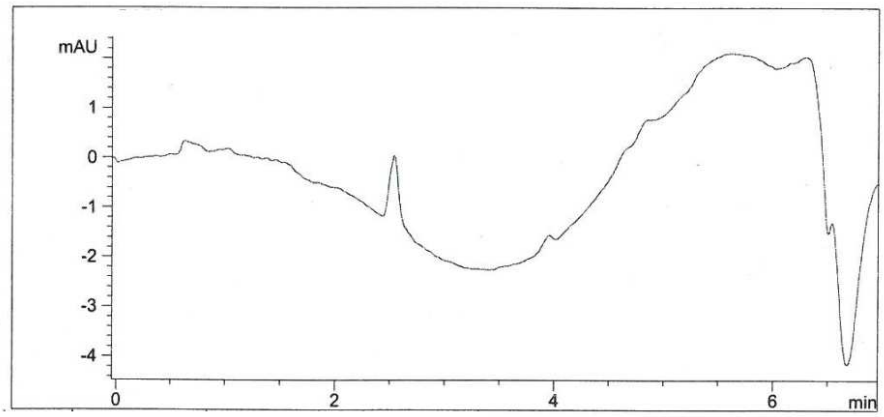

Figure 5. UV Report – Fraction 4

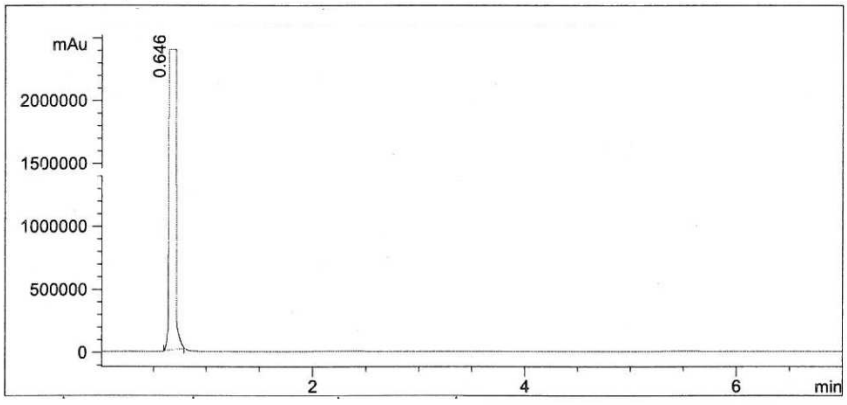

Figure 6. HPLC Report – Extracted Crude Sample

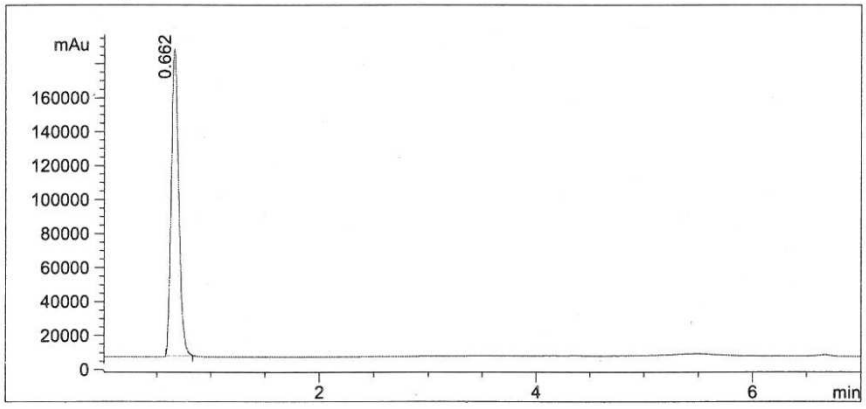

Figure 7. HPLC Report – Fraction 1

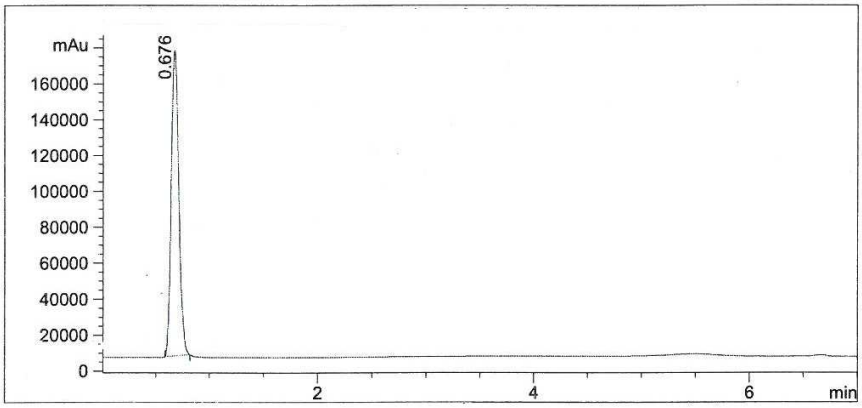

Figure 8. HPLC Report – Fraction 2

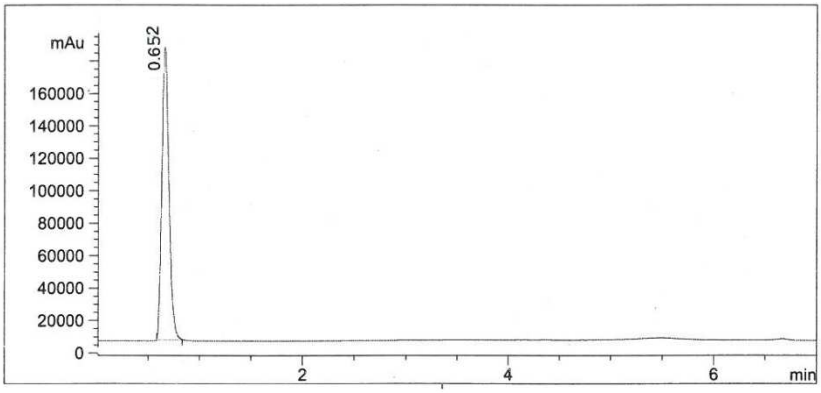

Figure 9. HPLC Report – Fraction 3

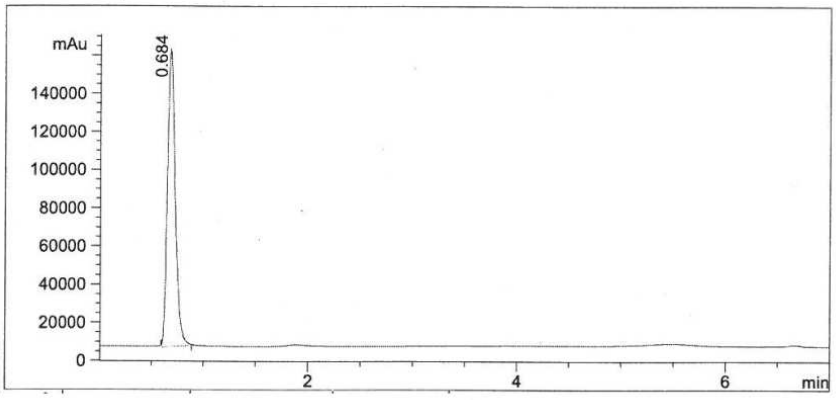

Figure 10. HPLC Report – Fraction 4

Mass report of crude and separated fractions

The Mass Spectrum detector gave the following spectrum for the Crude mixture at 0.575', fraction1 at 0.578', fraction2 at 0.593', fraction3 at 0.636 and 0.666' , fraction4 at 0.547 and 2.572'(Figures 11, 12, 13, 14, 15).

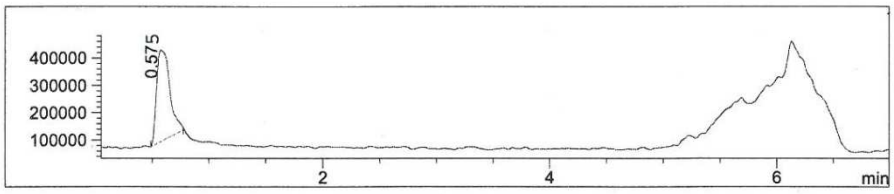

Figure 11. Mass Spectrum Detector Report – Crude Mixture

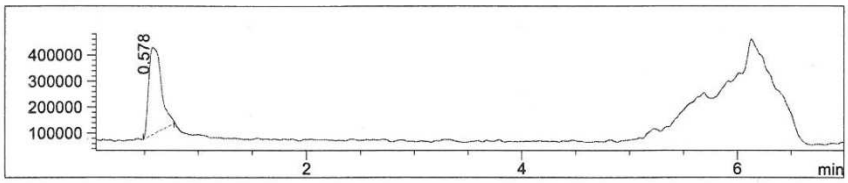

Figure 12. Mass Spectrum Detector Report – Fraction 1

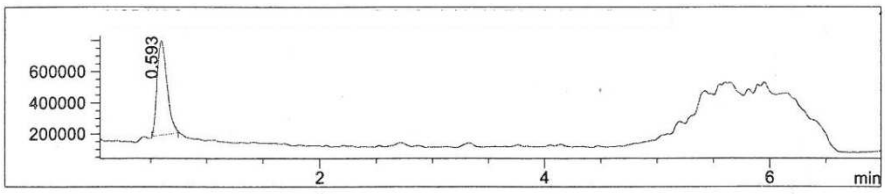

Figure 13. Mass Spectrum Detector Report – Fraction 2

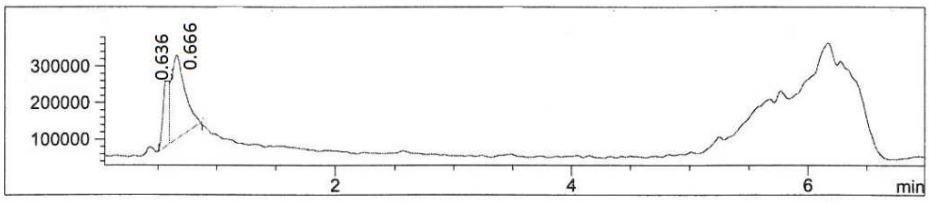

Figure 14. Mass Spectrum Detector Report – Fraction 3

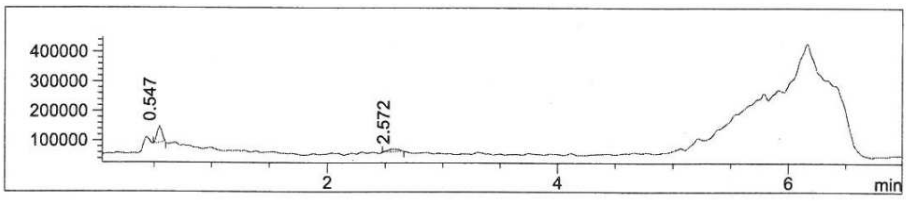

Figure 15. Mass Spectrum Detector Report – Fraction 4

The MS report recorded at the appropriate time as per MSD for crude mixture between time 0.493 : 0.732 gave the mass values 111.2, 126.9, 140.9, 163.0, 180.1, 202.9, 342.2, 365.0, 390.1, 391.2, 553.3 gives the conclusion the sugars may be monosaccharide and disaccharides with masses 180.1 and 342.2 and their fragments was shown in Figure 16, Fraction1 scanned between the time period 0.493:0.772 gave mass values 112.9, 145.1, 163.0, 164.1, 180.1, 202.9 which gives a conclusion that it could be a monosaccharide say glucose, fructose, galactose, mannose etc whose mass is 180.1 (Figure 17). Fraction2 scanned between the time period 0.507 : 0.745

gave mass values 111.2, 115.1, 140.9, 145.1,180.1, 198.0, 202.9 which gives a conclusion that it could be a monosaccharide say glucose, fructose, galactose, mannose etc whose mass are 180.1(Figure18). Fraction3 scanned between the time period 0.507 : 0.600 and 0.600 : 0.878 gave mass values 126.9, 163.0, 343.2, 360.0, 365.0, 374.0 and 126.9, 163.0, 342.2, 365.0, 375.1 respectively which concludes that, it may be a disaccharide say lactose, maltose, sucrose etc whose mass are 342.1 (Figure19 and 20). Fraction4 between the time period 0.493:0.600 and 2.482:2.667 gave mass 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 which concludes that it may be lactose, maltose, sucrose etc whose mass are 342.1 (Figures 21 and 22).

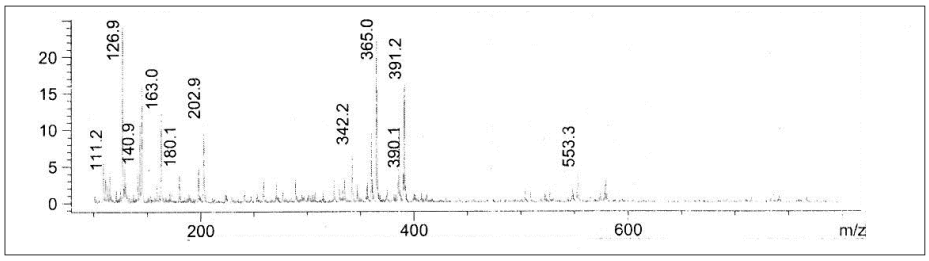

Figure 16. MS Report for the Crude Mixture (Time 0.493 : 0.732)

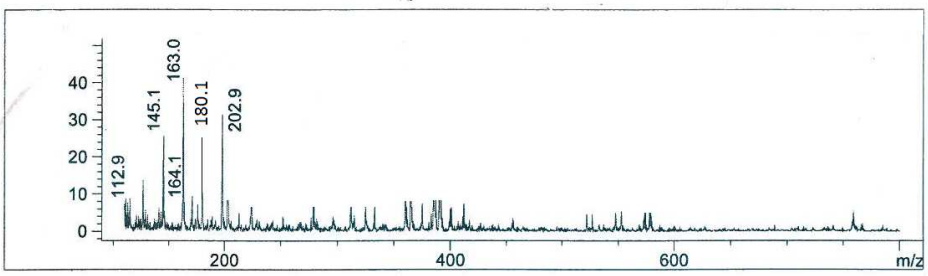

Figure 17. MS Report for the Separated Fraction1 (Time 0.493:0.772)

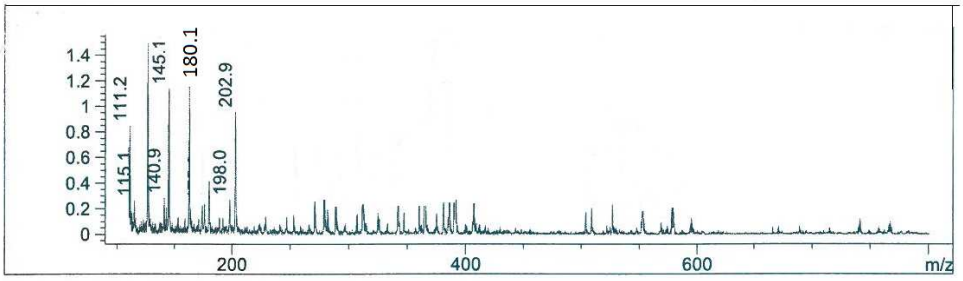

Figure 18. MS Report for the Separated Fraction2 (Time 0.507 : 0.745)

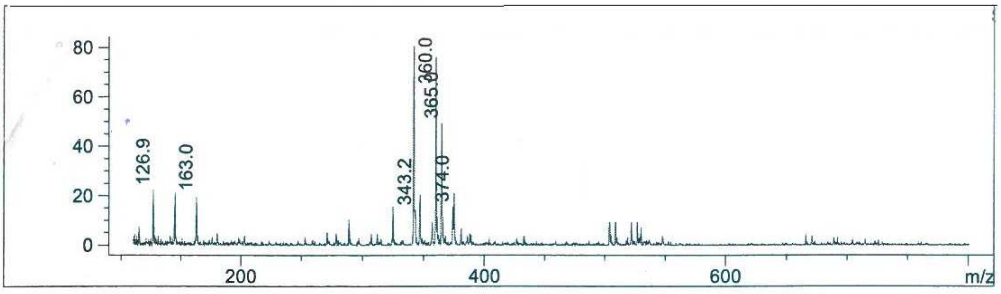

Figure 19. MS Report for the Separated Fraction3 (Time 0.507 : 0.600)

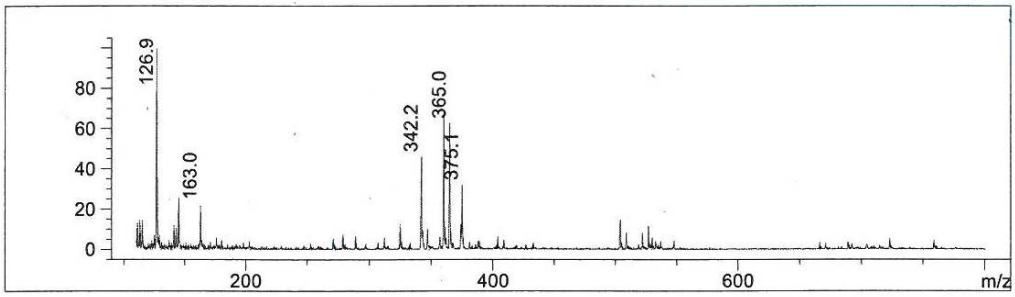

Figure 20. MS Report for the Separated Fraction3 (Time 0.600 : 0.878)

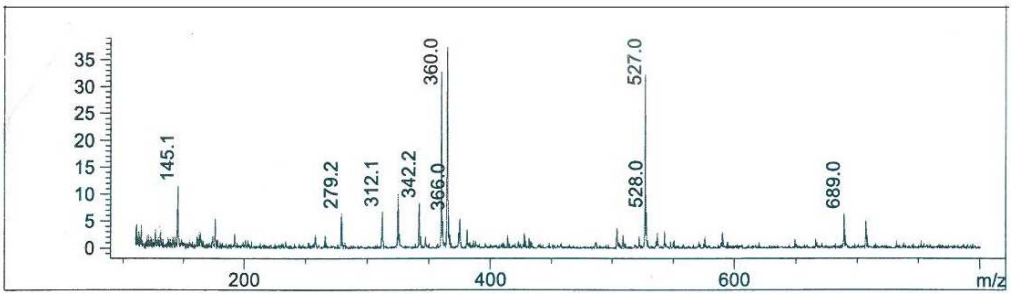

Figure 21. MS Report for the Separated Fraction4 (Time 0.493:0.600)

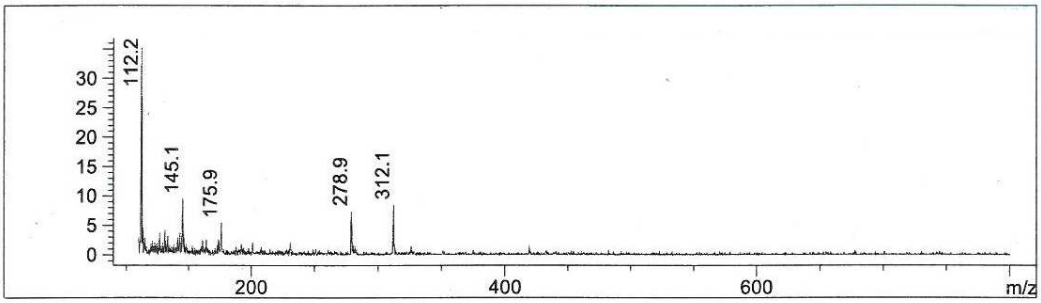

Figure 22. MS Report for the Separated Fraction4 (2.482:2.667)

Thin layer chromatographic analysis report

Four separated and purified sample fractions are spotted in the cellulose layer and the eluted species were mentioned as F 1, F 2, F 3, F 4 in the chromatogram was depicted in Figure 23

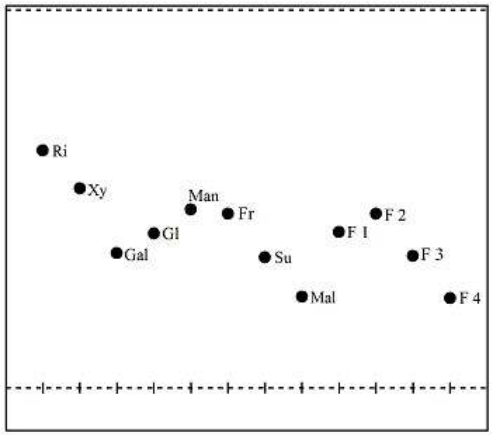

Figure 23 Developed thin layer chromatogram over a cellulose layer

(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 glucose, fructose, maltose and xylose. 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
Glucose	0.41	F1
Sucrose	0.35	F2
Fructose	0.46	F3
Maltose	0.24	F4
Xylose	0.53	-

CONCLUSION

These household solid wastes are of greater importance because the discarded portion is very high, for instance in the present work the non- edible portion of banana which is thrown is 20%. Therefore, there is often a serious waste disposal problem. A fruitful and economic industrial application was applied in this current work. Based on the above studies, we have developed a rapid method for the extraction of water soluble sugar. Briefly, dried ground sample is extracted with 80% hot ethanol followed by the usage of the mixture of Methanol - Dichloromethane – Water (0.3:4:1 v/v/v) which brings about much better results as compared with chloroform in the place of dichloromethane [10]. The advantage of using HPLC to measure sugars has been recognized for over a decade. HPLC has proven to be more selective than conventional wet methods, resulting in fewer samples pre-treatment. Additionally, HPLC allows individual quantification of several individual sugars in a single chromatographic run. Mass and TLC analysis gives accurate confirmation for the presence of glucose, fructose, sucrose and maltose. Keeping in view the results obtained, it may be concluded that banana peels possess good amount of nutritional value and rich in carbohydrates. Glass package and cold storage can retain good quality leading to more extensive shelf life of the products.

REFERENCES

[1] Leslie,S.; Cobley; From Longman Group Limited London. *An Introduction to the Botany of Tropical Crops,***1976**, 153.
[2] Akinyosoye, V.O; Macmillan Publishers Limited, *Tropical Agriculture*, **1991**, 65 – 68.
[3] Wath, J.N.; Brayer-Brand Wijk, M.G.; From E and S. Livingstone Ltd. Edinburgh and London, *The Medicinal and Poisonous Plants of South and Easthern Africa*, **1962.**
[4] Kudan M. J.;. From Parker Publishers Inc. Hattiesburg Maryland. *Encyclopedia of fruits, vegetables, nuts and seeds for healthful living*, **1962**
[5] A.,Sharaf, Ola.; A.;Sharaf, S.M.;Hegazi, Sedky. K.; *Chemical and biological studies on banana fruit*, Cairo university, **1979**, 18, 8-15.
[6] Baldwin, E;, Bell, D.J.; *Cole's Practical Physiological Chemistry*, published by Heffer, Cambridge, **1955**, 189.
[7] A .; Schweiger, *Journal of Chromatography*, **1962**, 9, 374.
[8] D. W.;Vomhot, T. C.; Tucher, *Journal of Chromatography* **1963**, 17, 300.
[9]M.; Lato, Brunelli, B.; G.; Ciuffins. *Journal of Chromatography*, **1968**, 34, 26.
[10] Pak S.; Chow, Simon M.; Landhäusser. *Tree Physiology* **2004**, 24, 1129 –1136