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Commercial Production of Simple Sugars from Calabash peels *(Lagenaria siceraria)* and identification using sophisticated Liquid chromatography/Mass Spectrometry & Thin Layer Chromatographic analysis

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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 - diethylamine - water (10:10:2:6, v/v/v/v) in the cellulose layer for TLC analysis which indicated the presence of lactose, sucrose, galactose and glucose. This is the first assay of the sugar profile of the bottle gourd peels, which can be further developed for characterization and evaluation of their quality with regards to their sugar composition.
Keywords: Sugar extraction; bottle gourd peels; UV inactive; Separation; LC/MS; TLC.

Introduction

It shares its common name with that of the calabash tree (*Crescentia cujete*). *Lagenaria siceraria* or *Lagenaria vulgaris*, the calabash, bottle gourd, opo squash or long melon is a vine grown for its fruit, which can either be harvested young and used as a vegetable, or harvested mature, dried, and used as a bottle, utensils such as cups, bowls, and basins in rural areas. For this reason, the calabash is widely known as the bottle gourd[1]. The fresh fruit has a light green smooth skin and a white flesh. The rind of the domesticated calabash, unlike that of its wild counterpart, is thick and waterproof. Rounder varieties

are called calabash gourds. They come in a variety of shapes, they can be huge and rounded, or small and bottle shaped, or slim and more than a meter long. The calabash was one of the first cultivated plants in the world, grown not primarily for food, but for use as a water container. The bottle gourd may have been carried from Africa to Asia, Europe and the Americas in the course of human migration[2]. The larger-fruiting varieties whose fruits are used mostly for making containers or other handicrafts and the smaller-fruiting variety whose fruits are more edible. In some Caribbean countries, it is worked, painted and decorated as shoulder bags or other items by artisans, and sold to tourists. Calabashes are used in making the *shegureh* (a Sierra Leonean women's rattle) and *balangi* (a Sierra Leonean type of *balafon*) musical instruments. Sometimes, large calabashes are simply hollowed, dried and used as percussion instruments [3]. Hindu ascetics (*sadhu*) traditionally use a dried gourd vessel called the *kamandalu*. The calabash, as a vegetable, is frequently used as either a stir-fry or in a soup[4]. The juice is considered to have many medicinal properties and very good for health. Additionally, the gourd can be dried and used to smoke pipe tobacco. The present investigation is concentrated mainly on the water soluble sugars present in the peels of bottle gourd which have wide application in industries and most economical process.

Experimental

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 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 [5-7].

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. Extracted Sample was 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 [5-7].

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 - 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 [8-11]. After the elution, plate was dried under warm air. The plate was sprayed with 5% diphenyl - amine 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

Analysis report showed that the extracted separated components are UV inactive as in Figure 1.

The Mass Spectrum detector gave the following spectrum of fraction 1 at 0.636 and 0.666 min, fraction 2 at 0.525 and 0.702min, fraction3 at 0.606 and 2.637min, fraction4 at 0.595 and 2.576min. The MS report recorded at the appropriate time as per MSD for Fraction 1 scanned between the time period 0.507:0.600min gave m/z values 126.9, 163.0, 343.2, 360.0, 365.0, 374.0 and 0.600 : 0.878 min gave m/z values 126.9, 163.0, 342.2, 365.0, 365.0, 375.1. Fraction 2 scanned between the time periods 0.480: 0.546 min gave m/z values 115.1, 145.1, 175.9, 279.2, 312.1, 366.0, 365.0, 707.2 and 0.573: 0.812 min gave m/z values 111.2, 145.1, 279.2, 312.1, 360.0, 365.0, 707.2. Fraction 3 scanned between the time period 0.507: 0.798 min and 2.495: 2.760min gave m/z values 112.9, 145.1, 163.0, 180.1, 198.0, 360.0 and 112.1. Fraction 4 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 respectively depicted in Figure 2-5.

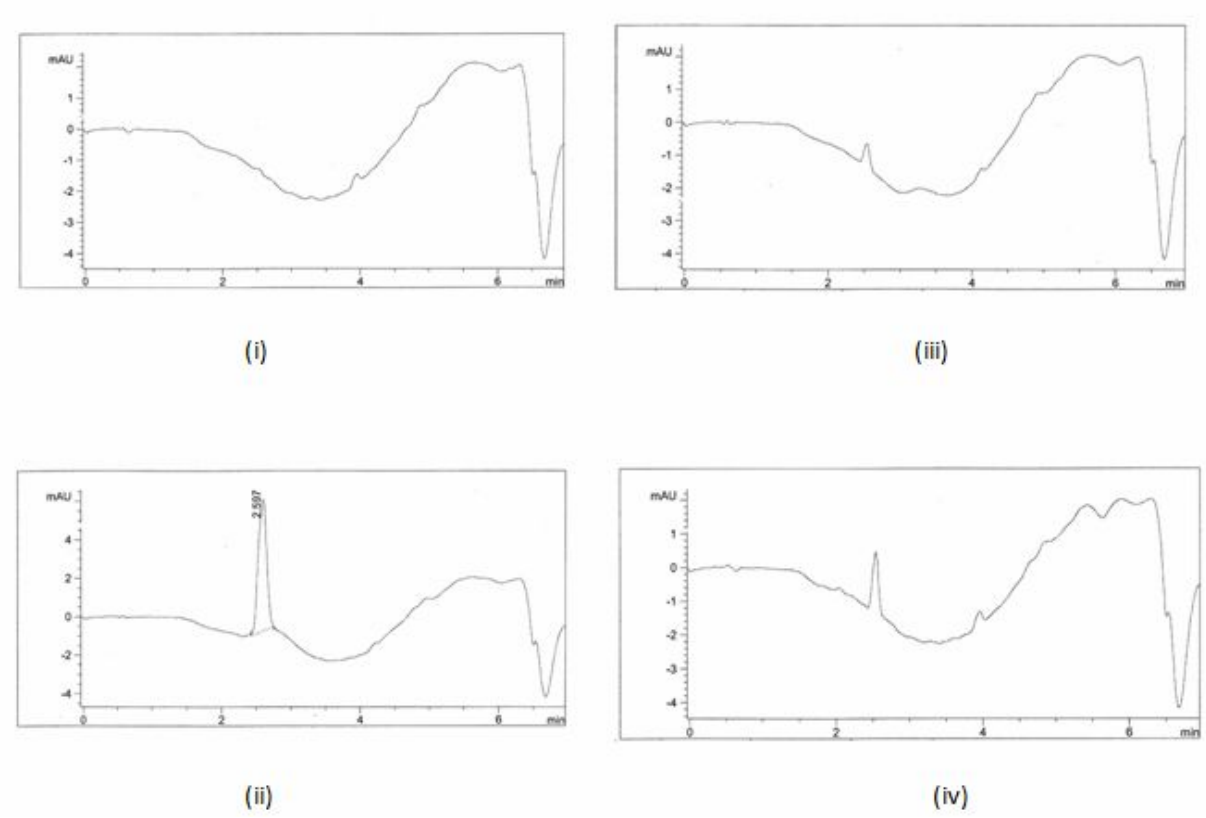

Figure 1. UV inactive spectrum of the Separated Fractions

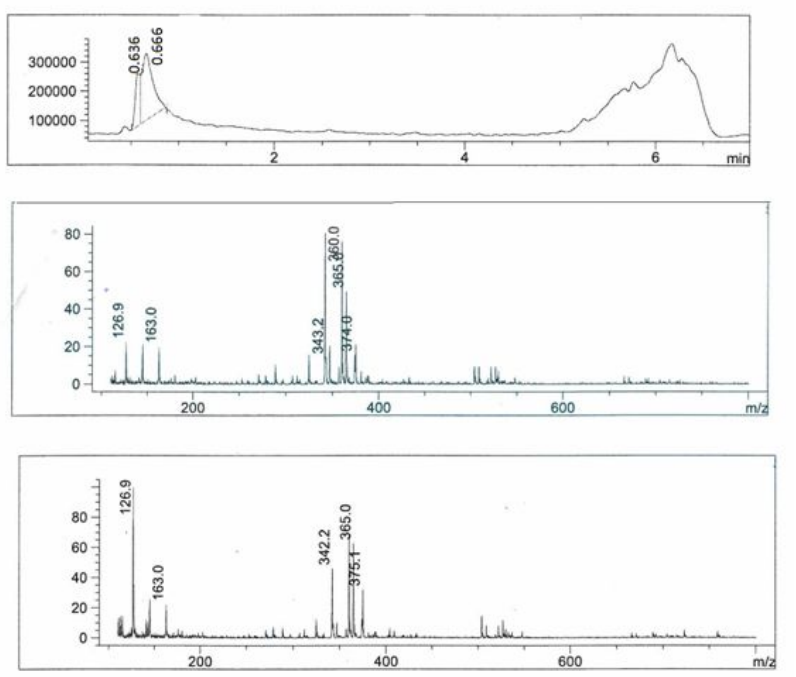

Figure 2. Mass report of Separated Fraction 1

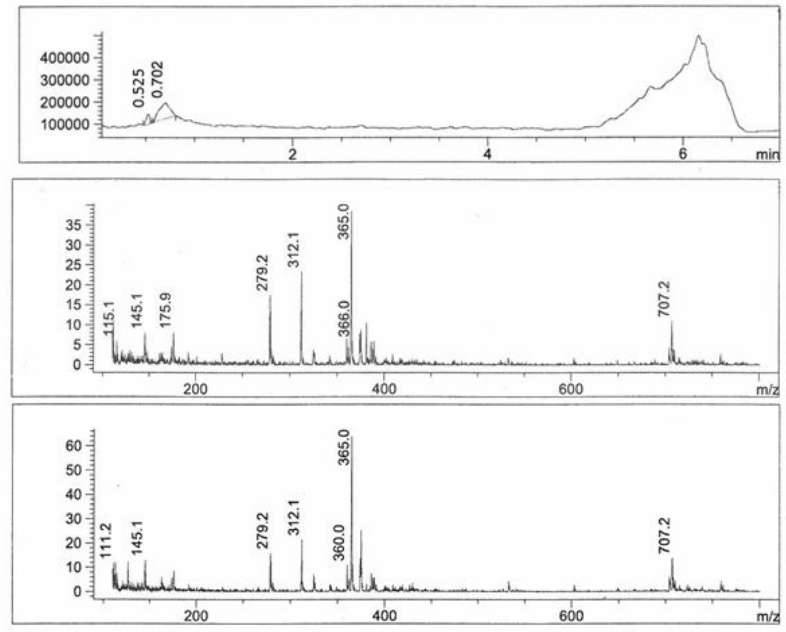

Figure 3. Mass report of Separated Fraction 2

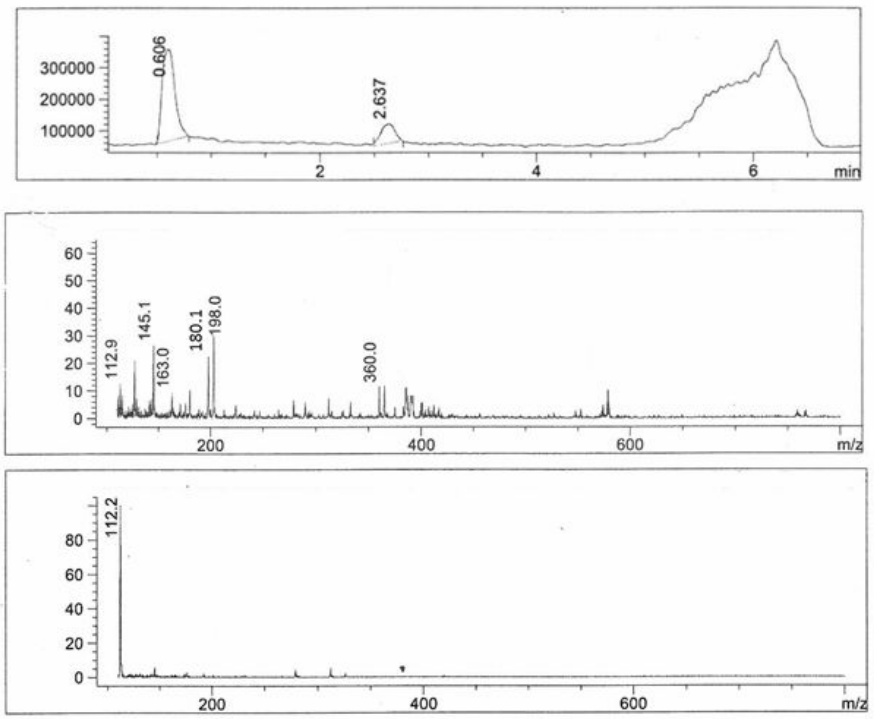

Figure 4. Mass report of Separated Fraction 3

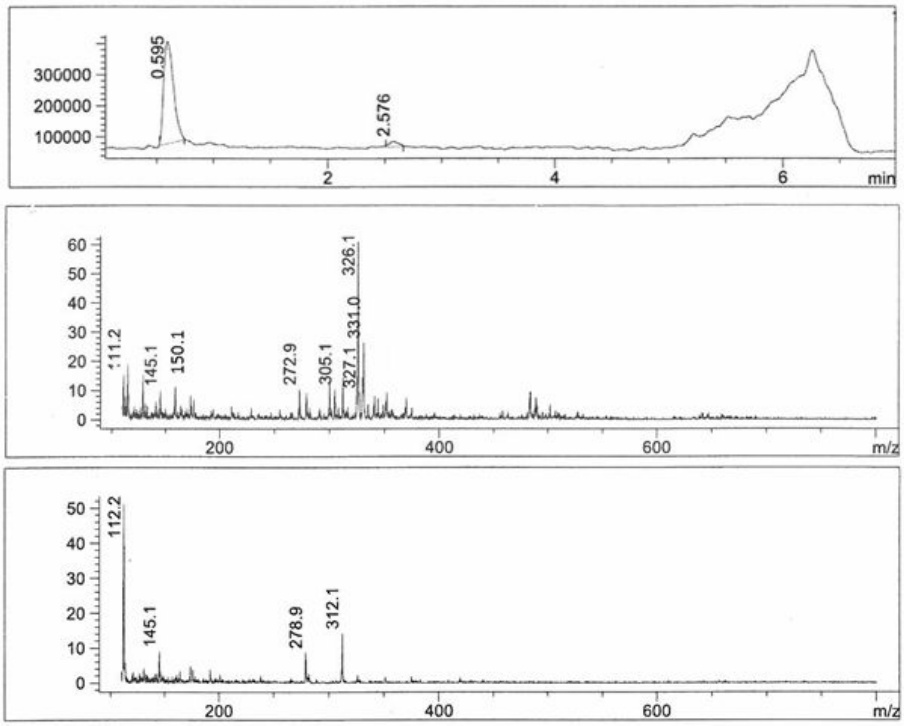

Figure 5. Mass report of Separated Fraction 4

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, F4 in the chromatogram shown in Fig 6. The fractions obtained were found to be matching with four standard sugars. R_f value for the analytical grade samples shown in Table 1.

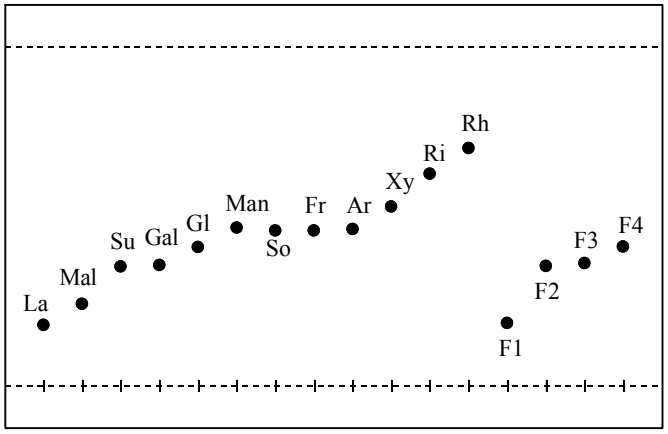

Figure 6. Developed thin layer chromatogram over a cellulose layer, (La – Lactose, So – Sorbose, Ar-Arabinose, Rh – Rhamnose, Ri – Ribose, Xy-Xylose, Gal – Galactose, Gl - Glucose, Man – Mannose, Fr - Fructose, Su – Sucrose and Mal –Maltose).

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	0.18	F1
Maltose	0.24	-
Sucrose	0.35	F2
Galactose	0.36	F3
Glucose	0.41	F4
Mannose	0.47	-
Sorbose	0.46	-
Fructose	0.46	-
Arabinose	0.46	-
Xylose	0.53	-
Ribose	0.63	-
Rhamnose	0.70	-

Conclusions

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, galactose, sucrose and xylose.

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