

## Monosaccharide profile of “*Mendim Me Zon*”: Beverage from *Solanum aethiopicum* Shum berries

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### Abstract

*Solanum aethiopicum* Shum is a plant belonging Solanaceae family. These berries are used for their food and pharmacological virtues. For instance, they are commonly consumed in the form of hot-drink, so called “*Mendim Me Zon*” used by the forest people. Revitalizing and detoxifying properties are attributed to this beverage. The bioactivity of this drink is often attributed to its secondary metabolites. While monosaccharides also have a biological activity that may contribute to the virtues of this tea. The present work was carried out to analyse the carbohydrate fraction of “*Mendim Me Zon*”. The extract prepared following the preparation conditions of “*Mendim Me Zon*” was analysed to determine its proximate composition, including soluble carbohydrates, proteins and total phenolic compounds. The monosaccharide profile was established using gas chromatography. Results show that carbohydrates were its most abundant constituent in the extract, although the taste was bitter. This taste is certainly determined by phenolic compounds present in the extract. Gas chromatography analysis of the extract showed that extract was composed of pentose (fucose), hexoses (mannose, galactose and glucose), hexosamines (glucosamine and galactosamine), uronic acid (glucuronic acid) and sialic acid (N-acetyl-neuraminic acid). Fructose was absent from the extract, while the mannose was relatively more abundant. These results might suggest that “*Mendim Me Zon*” contains monosaccharides and their derivatives with many beneficial biological functions. The monosaccharide profile justifies the traditional use of this drink as a nutraceutical. However, further studies including toxicological one are still needed to determine the safety and efficacy of “*Mendim me Zon*”.

**Keywords:** *Solanum aethiopicum* Shum berries; *Mendim Me Zon*; Gas chromatography; Monosaccharide profile

### 1. Introduction

*Solanum aethiopicum* Shum is an herbaceous plant, belonging to the Solanaceae family, which can 1.8 m in height in favourable ecological conditions [1]. It adapts to various climate, including the driest. The leaves are alternates, lobed, 20 to 30 cm long, and are fairly widely spaced. The stems are strong and dark coloured, the roots brown with a length of 25 cm and a thickness of 10 mm. The white hermaphroditic flowers are small and grouped by 5 to 8 on the stem between two leaf stages. The round berries are grouped in clusters on one side of the stem, and their colour changes from green to red, to orange when they ripen. Their diameter varies between 1 and 2 cm, the flat seeds, about 3 mm long, are dispersed in the pulp of the berry. The seeds can retain their germinated power 6 to 12 months after drying. This plant resists to diseases affecting other species of *Solanum* gender [2]. It is widespread in Central, West and

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Southern Africa, popular in Cameroon, Nigeria and even more so in Uganda, where it is grown in the swamps during the dry season [3].

Previous studies have been established its functional and therapeutic virtues of *S. aethiopicum* Shum berries. The antiviral properties of these berries have highlighted one of the types of HIV [4]. Adeyeye and Adanlawo [5] emphasised the amino acid profile of the berries of *S. aethiopicum* Shum. Our present studies have shown that the consumption of *S. aethiopicum* berries enhances the physical endurance in rats [6]; and prevents the occurrence of metabolic and inflammatory syndromes [7].

Nevertheless, the drink is preferred to raw berries. Its preparation is simple and straightforward. It consists of immersing the washed berries in boiled water and crushed them using a wooden spatula, then filtering with a kitchen sieve. Other ingredients may be added in certain cases, for reasons of flavour or care of certain diseases [8]. The filtrate (beverage) so called locally "*Mendim Me Zon*", is served hot and drunk plain, with a tuber or plantain as an accompaniment. Revitalizing and detoxifying properties are also attributed to this beverage. The virtues of this traditional tea can be induced by their bioactive substances such as amines, minerals, vitamins, glycoalkaloids and phenolic compounds [2, 9]. Previous studies have proven that monosaccharides and their derivative compounds such as glucosamine, galactosamine, sialic acid and glucuronic acid are involved in various biological functions [10, 11, 12, 13]. However, to the best of our knowledge, no study has been conducted to establish the monosaccharide profile of "*Mendim Me Zon*". Thus, the present study was carried out to analyse the carbohydrate fraction of "*Mendim Me Zon*".

## 2. Material and methods

### 2.1. Preparation of extract

The *S. aethiopicum* Shum (SAS) berries have been harvested in the locality of Ambam in the southern region, Cameroon, Central Africa. In the different laboratories, they were washed and treated with a 2% of sodium hypochlorite solution. Berries were sliced in quarters, then dried with an oven at 40°C and grinded as described previously [7]. The extract was prepared by immersion of the powder of SAS berries in boiling water for 5 minutes. The mixture was filtered with a wattman paper, the obtained filtrate was lyophilized and retained in the freezer until the analyses.

### 2.2. Proximate composition of extract

The protein content of extract was determined using the method by Lowry et al. [14]. The carbohydrates available from the extract were measured using the method described by Fischer and Stein [15]. The total phenolic compounds were extracted in 70% ethanol and tested by the method of Marigo [16] using the reagent of Folin - Ciocalteu.

### 2.3. Analysis of extract by gas chromatography

The glycosylated fraction of "*Mendim Me Zon*" was analysed by identification of its monosaccharides by gas chromatography coupled to a flame ionization detector (GC/FID) using two derivation methods: the HFB derivation with heptafluorobutyric anhydride (HFB) and the trimethylsilyl (TMS) bypass-TMS. The identification was also carried out by gas chromatography coupled with mass detection and electron impact ionization (GC/MS-EI). The qualitative analysis of "*Mendim Me Zon*" monosaccharides was based on the variation of the relative abundance as a function of the m/z ratio and the retention times of the derived samples subjected to gas chromatography under two types of detection: spectroscopy of mass-electron impact ionization and flame ionization, with lysine as internal standard.

#### 2.3.1. Monosaccharides identification by GC/MS-EI after heptafluorobutyric acid derivation

The derivation - HFB was carried out by adding 100 µL of anhydrous acetonitrile (dried in calcium chloride) and 20 µL of heptafluorobutyric anhydride (HFBA) using respectively 200 µL and 20 µL pipettes, to which a small hose end with a glass Pasteur pipette was added. The sample was heated for 15 min to 180°C. In a sand bath, then evaporated to dryness under nitrogen and taken up with 100 µL of acetonitrile.

#### 2.3.2. Monosaccharides identification by GC/MS-EI after trimethylsilyl derivation

The derivation - TMS, N-reacetylation was first carried out for the extraction of amino sugars (Galactosamine, Glucosamine, Mannosamine...) after cooling, the solution was neutralized by addition of Ag<sub>2</sub>CO<sub>3</sub> (a tip of a spatula from the powder) to pH 6-7 and N-reacetylation the mixture by adding 20 µL of acetic anhydride. The mixture was left overnight at room temperature in the dark. After centrifugation, the supernatant was collected in a cut and sealed Pasteur pipette.

The fatty acids were extracted three times with heptane (3 x 250 µL). The solution was evaporated to dryness under nitrogen. The derivatization was carried out by adding 20 µL of pyridine and the same volume of anhydrous bis(trimethylsilyl) trifluoroacetamide (BSTFA), the monosaccharides were derived for 2 h at room temperature. The derived samples were analysed by gas chromatography coupled with mass spectrometry – electron impact ionization [Trace GC Ultra from Thermo Scientific, TSQ Quantum GC triple quadrupole mass detector from Thermo Scientific Column SGE Forte SolGel-1 ms (30 m x 0.25 µm), 100% dimethyl polysiloxane on a SolGel matrix, nonpolar, at 0–380°C]. The same samples were also analysed by gas chromatography coupled to a flame ionization detector [Trace GC Ultra from Thermo Electron, Alltech Econo-Cap EC-1 column (30 m x 0.25 mm, 0.25 µm)].

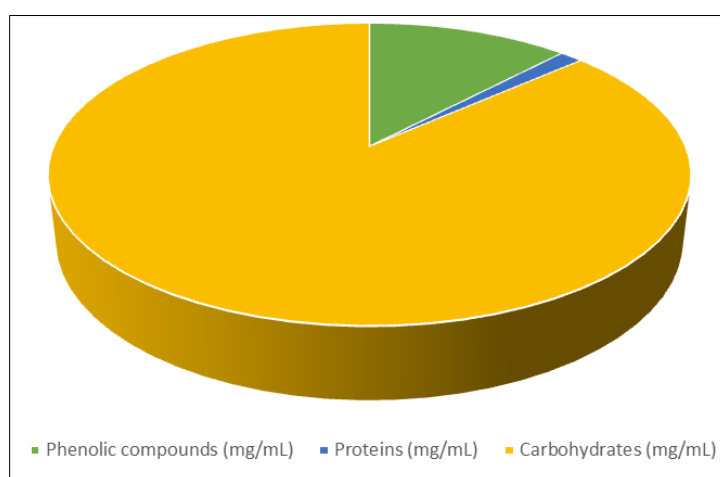
The following parameters were common to both methods: Ross injector temperature (250°C); injector pressure (100 kPa); carrier gas (helium); source of temperature (250°C); interface temperature (280°C); electron impact ionization (EI); mass range (50–1000 µg); scan time (0.400 ms); current (25 µA); resolution Q1 (0.70 FWHM); filament lit from 3 min and ionization energy (70 eV).

A volume of 1 µL of sample was injected for the GC/MS EI-HFB and GC/MS EI – TMS methods. EI-Sugar HFB followed the temperature program: 100°C – 140°C (1.2°C/min) – 240°C (4°C/min), then plateau at 240°C for 10 min, the analysis lasted 68.33 min. EI\_Sugars TMS was carried out according to the temperature program: 120°C – 240°C (2.0°C/min), then plateau at 240°C for 10 min. The analysis lasted 70 min. A volume of 0.5 µL of sample was injected for the GC/FID-HFB and GC/FID-TMS methods. The GC/FID, HFB method followed the temperature program: 100°C – 140°C (1.2°C/min) – 240°C (4°C/min), then plateau at 240°C for 10 min. The analysis time was 68.33 min; Ross injector temperature (300°C); and the FID temperature (250°C, hydrogen). The GC/FID, TMS method followed the temperature program: 120°C – 240°C (2.0°C/min), then the plateau at 240°C for 10 min. The analysis time was 70 min; Ross injector temperature: 300°C and FID temperature: 260°C (hydrogen). The monosaccharides were identified by correspondence with the specific ions and the masses extracted by electron ionization impact.

### 3. Results and discussion

#### 3.1. Proximate composition of extract

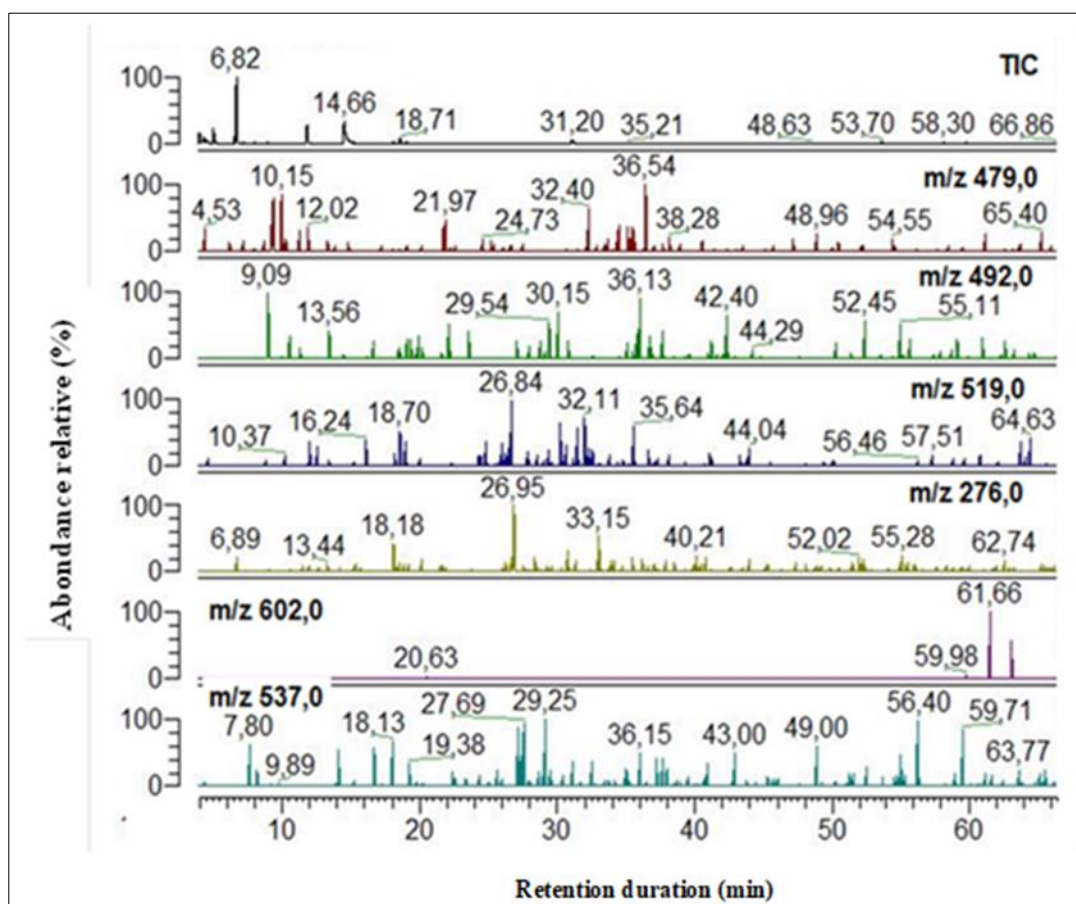
The protein, phenolic and carbohydrate contents of the extract of *Solanum aethiopicum* Shum are presented in figure 1. The extract consisted mainly of carbohydrates, followed by polyphenols. Chinedu et al. [17] reported a similar trend when studying the proximate composition of whole berries of *Solanum aethiopicum*. Proteins were low while lipids absent. This composition raises questions when one knows that “Mendim Me Zon” has a bitter taste. Given this chemical composition of the extract, it was expected to have a sweet taste. However, the taste of the aqueous extract was bitter. This taste would be determined by the secondary metabolites of the extract. While soluble carbohydrates were in the majority. How could this extract not be sweet? Only the nature of the monosaccharides that make up the extract can explain this.



**Figure 1** Proximate composition of the extract *Solanum aethiopicum* Shum

### 3.2. Identification of monosaccharides and their derivatives groups

Gas chromatography coupled with mass spectrometry with electron impact or flame ionisation detector after the derivation of heptafluorobutyric acid was used to analyse the glycoproteins of *Balanites aegyptiaca* fruits [18]. Figure 2 shows the comparison of chromatograms (GC/MS-EI) of extract in function of m/z signal and retention time, after derivation of heptafluorobutyric (HFB). Mass to charge ratio (m/z) was used to identify the monosaccharides and their derivatives from *Solanum aethiopicum* Shum extract. M stands for mass and z stands for charge number of ions. In mass analysis, an electron taken from molecules to create single charge ions. If two electrons are removed, double charged ions are produced. The number of electrons removed is the number of charge. Mass/charge (m/z) represents mass divided by charge number and the horizontal axis in a mass spectrum is expressed in units of m/z. Since z is almost always 1 with GC/MS, the m/z value is often considered to be a mass. These results indicate that following monosaccharide and their derivative groups have been revealed in function of pentoses, hexoses, deoxyhexoses, uronic acids and sialic acids in the aqueous extract of *Solanum aethiopicum* Shum berries. It is in like manner that m/z (479.0) corresponds to pentoses; m/z (492.0) to deoxyhexoses; m/z (519.0) to hexoses; m/z (276.0) to hexoamines; m/z (537.0) to uronic acid, and m/z (602.0) to sialic acid. The presence of this sialic acid in the extract of the *Solanum aethiopicum* Shum berries suggests the existence of glycolipids in the extract. Numerous works have shown that the proteolytic enzymes of plant origin are glycoproteins [19, 20, 21]. N-acetyl neuraminic acid will be a barrier to pathogenic microorganism [22].

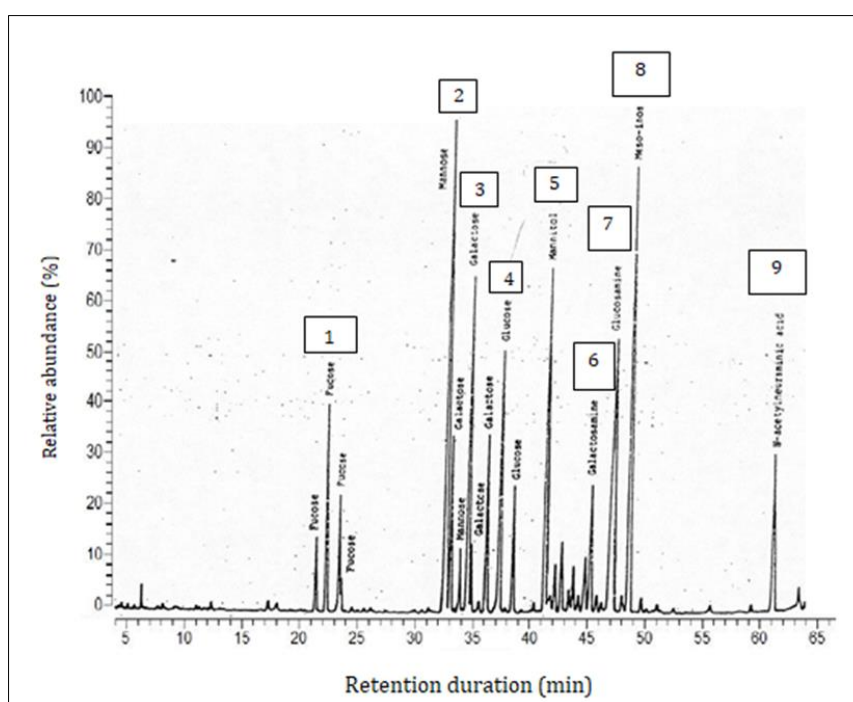


**Figure 2** Comparison of chromatograms GC/MS-EI of *S. aethiopicum* Shum extract in function of m/z signal and retention time after HFB derivation. The mass detector was a triple quadrupole (TSQ Quantum gas chromatography of Thermo Scientific)

The stationary phase was non polar with the dimethylpolysiloxane in a SolGel matrix. Lysine was used like internal standard. m/z (479.0) = pentoses; m/z (492.0) = deoxyhexoses; m/z (519.0) = hexoses; m/z (276.0) = hexoamines; m/z (537.0) = uronic acids; m/z (602.0) = sialic acid.

### 3.3. Monosaccharides profile of the extract of *Solanum aethiopicum* Shum

The trimethylsilyl derivation has usually helped in amplifying the detection of monosaccharides or their derivatives in plant extracts [23]. The monosaccharides profile of *Solanum aethiopicum* Shum extract after trimethylsilyl derivation is presented in the figure 3. Chromatogram shows that we can find four monosaccharides in the extract, including fucose, mannose, galactose and glucose. The relative abundance of mannose was higher than that of galactose, glucose and fucose. Five derivatives of monosaccharides have been found in the extract of *S. aethiopicum* Shum. According to different standards, Fucose in the extract was noted with m/z (492.0) and at retention duration of 24.6 min. Mannose was detected after extraction of the m/z (519.0) ion corresponding to a retention time of 32.84 min; with the same m/z galactose and glucose were detected at 34.18 and 37.72 min respectively. Galactosamine and glucosamine were detected after extraction of the m/z (276) ion corresponding to retention duration of 45.34 and 47.58 min respectively. N-acetyl neuraminic acid was identified in the aqueous extract of the *Solanum aethiopicum* Shum berries with a retention time of 61.66 min in function of m/z (602.0). The derivatives of monosaccharides found in *S. aethiopicum* Shum extract were galactosamine, glucosamine, mannitol, meso-inositol and N-acetyl nuramminic acid. Monosaccharides are known for their energetic function. However, recent studies have shown that some monosaccharides and their derivatives have important biological activities [6, 7]. Moreover, several authors reported bioactivity of many monosaccharides and their derivatives. Hepatotoxicity has been associated to galactosamine [11]. Cytotoxicity activity of fucose [12] can be used to fight against cancer. In contrast, the cytoprotective function of glucosamine has been proven [10]. N-acetyl neuraminic acid possess immune proprieties [13].



1: Fucose, 2: Mannose, 3: Galactose, 4: Glucose, 5: Mannitol, 6: Galactosamine, 7: Glucosamine, 8: Meso-inositol, 9: N-acetyl neuraminic Acid.

**Figure 3** Chromatogram GC/MS-EI of *S. aethiopicum* Shum extract after TMS derivation (Trace GC Ultra de Thermo Electron). Column Alltech Econo-Cap EC-1 (30 m × 0.25 mm, 0.25 μm).

### 4. Conclusion

“*Mendim Me Zon*” is traditional beverage made with *S. aethiopicum* shum berries; used by forest people as nutraceutical. This study was performed to analyse the carbohydrate fraction of “*Mendim Me Zon*” by gas chromatography. Results showed that carbohydrates were the most abundant component in the beverage, despite of its bitter taste. Gas chromatography analysis of beverage revealed that it was composed of monosaccharides and its derivatives, including fucose, mannose, galactose, glucose, glucosamine, galactosamine, glucuronic acid and N-acetyl-neuraminic acid. Fructose was absent from the beverage, while the mannose was relatively more abundant. These results might suggest that “*Mendim Me Zon*” contains monosaccharides and their derivatives with many beneficial biological functions. The monosaccharide profile justifies the traditional use of this drink as a nutraceutical. However, further studies including toxicological one is still needed to determine the safety and bioactivity of “*Mendim me Zon*”.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors declare that there is no conflict of interest regarding these results.

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